

Docket No.: SER-001
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Seymour Fein

Application No.: 10/706,100

Confirmation No.: 7710

Filed: November 12, 2003

Art Unit: 1654

For: Pharmaceutical compositions including low
dosages of desmopressin

Examiner: A. D. Kosar

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF

This Appeal Brief under 37 C.F.R. § 41.37 is accompanied by a petition for a four month extension of time and an authorization to charge the petition fee and the fee set forth in 37 C.F.R. § 41.20(b)(2) to Deposit Account No. 07-1700. Although Appellants believe no further fee is due, please also charge any other required fee to Deposit Account No. 07-1700.

I. REAL PARTY IN INTEREST

The real parties in interest are 1) the assignee, Reprise Biopharmaceutics, LLC, by virtue of an assignment from the inventor, as recorded at Reel 021121, Frame 0562, and 2) its exclusive licensee, Serenity Pharmaceuticals Corporation.

II. RELATED APPEALS AND INTERFERENCES

This application is a continuation-in-part of international patent application PCT/US03/14463, filed on May 6, 2003, claiming the benefit of Great Britain application 0210397.6.

There are no pending appeals or interferences known to be related to the present appeal. Appellant currently intends to appeal the final rejection of U.S. Application 12/173,072, a continuation of the present application.

A division of the present application, directed to methods of using compositions of the type claimed herein, has issued as U.S. Patent No. 7,405,203. A continuation of that division, again directed to methods of using the compositions, has issued as U.S. Patent No. 7,579,321. Copies are attached as Exhibits A and B.

III. STATUS OF CLAIMS

Claims 1-33 have been presented in the present application. Of these, claims 2, 5, 8 and 10-26 have been canceled. As such, claims 1, 3-4, 6-7, 9 and 27-33 remain pending.

Claims 1, 3-4, 6-7, 9 and 27-33 stand rejected and Appellant appeals the rejection of these claims.

A copy of the rejected claims in the present appeal is provided in the claims appendix.

IV. STATUS OF AMENDMENTS

An amendment to the claims was filed under 37 C.F.R. § 1.116 on February 10, 2009. The amendment was not entered, as indicated in the Advisory Action of March 12, 2009. As such, the claims remain as presented on April 8, 2008.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Desmopressin is an analogue of the hormone vasopressin, and has been “prescribed for voiding postponement, incontinence, primary nocturnal enuresis, and nocturia” (Application, p. 1, lines 20-26). However, “standard dosages of desmopressin have been shown to cause undesirable side-effects including high incidences of hyponatremia” (Application, p. 2, lines 21-23). Some patients receiving conventional desmopressin therapy have suffered hyponatremic-related *seizures*; in two cases, *the patient died*. See FDA Notice: Information for Healthcare Professionals: Desmopressin Acetate (marketed as DDAVP Nasal Spray, DDAVP Rhinal Tube, DDAVP, DDVP, Minirin, and Stimat Nasal Spray), a copy of which is attached as Exhibit C, at p. 3. The FDA has therefore warned that conventional intranasal desmopressin “formulations are no longer indicated for the treatment of primary nocturnal enuresis” (FDA Notice, p. 1).

The claimed invention provides novel pharmaceutical compositions and dosage forms that address the serious safety concerns of prior art desmopressin dosage forms. Specifically, dosage forms that induce a lower peak desmopressin concentration in a patient’s bloodstream provide the therapeutic antidiuretic effects of desmopressin with excellent safety and tolerability (Application, Example 8). Desmopressin administration achieving a peak blood concentration that does not exceed 10 picograms per mL is novel and inventive over the prior art. See, for example, the Notice of Allowance in related U.S. Patent No. 7,405,203:

The following is an examiner’s statement of reasons for allowance:

As amended above, the claims are distinguished over the art, as the art did not recognize achieving a C_{max} of 10 pg/ml or less. For example, BENGTTSSON (US Patent 5,763,398; PTO-1449, 5/4/07) teaches achieving a C_{max} of approximately 400 pg/ml, however there is no teaching or suggesting in Bengtsson, or in combination with any other art of record, to lower the C_{max} to 10 pg/ml. (Exhibit D, pp. 4-5)

The claimed pharmaceutical compositions and dosage forms are effective because Appellant discovered “desmopressin can produce this essential antidiuretic effect at much lower doses and lower blood concentrations than previously thought” (Application, p. 37, lines 9-11). Dosage forms that establish a lower peak concentration of desmopressin permit better control over the period of antidiuresis. As a result, urine production can resume at an appropriate time and hyponatremia (and its dangerous consequences) can be avoided.

Appellant has described and claimed dosage forms that decouple, for the first time, the important, therapeutic benefits of desmopressin from the dangers of conventional, prior art desmopressin formulations.

Claim 1

The invention of claim 1 is directed to a pharmaceutical composition comprising desmopressin and a pharmaceutically acceptable carrier¹ ***in a dosage form adapted*** for intranasal, transdermal, or intradermal administration² ***sufficient to establish in a patient a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum***³ and to decrease urine production.⁴ The pharmaceutical composition comprises 0.5 ng to 20 µg of desmopressin.⁵

Claim 4

The invention of claim 4 is directed to a pharmaceutical composition according to claim 1 that comprises from about 0.1 µg to about 2 µg of desmopressin.⁶

Claim 6

The invention of claim 6 is directed to a pharmaceutical composition according to claim 1 in a dosage form adapted for transdermal delivery and comprising a patch, gel, cream, ointment, or iontophore.⁷

Claim 7

The invention of claim 7 is directed to a pharmaceutical composition according to claim 1 adapted for intradermal administration comprising a patch.⁸

¹ Described in the application at, for example, page 4, lines 2-3.

² Such dosage forms are described in the application at, for example, page 24, lines 19-27, and in the table on page 25.

³ Described in the application at, for example, page 4, lines 4-8.

⁴ Described in the application at, for example, page 25, lines 1-2, and from page 36, line 13, to page 37, line 12.

⁵ Described in the application at, for example, page 4, lines 2-3.

⁶ Described in the application at, for example, page 6, lines 20-21, and in the table on page 25.

⁷ Such dosage forms are described in the application at, for example, page 24, lines 19-27, and in the table on page 25.

Claim 9

The invention of claim 9 is directed to a pharmaceutical composition according to claim 1 in a dosage form sufficient to establish in a patient a steady plasma/serum desmopressin concentration in the range of *from about 0.5 picograms desmopressin per mL plasma/serum to about a maximum of 5.0 picograms desmopressin per mL plasma/serum*.⁹

Claim 27

The invention of claim 27 is directed to a pharmaceutical dosage form comprising desmopressin and a pharmaceutically acceptable carrier¹⁰ *adapted for intranasal administration*¹¹ *which* when administered to a patient *establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum*¹² *for a time between four and six hours* and decreases urine production¹³.

Claim 28

The invention of claim 28 is directed to a pharmaceutical dosage form according to claim 27 that establishes a steady plasma/serum desmopressin concentration in the range of *from about 0.5 picograms desmopressin per mL plasma/serum to about a maximum of 5.0 picograms desmopressin per mL plasma/serum*.¹⁴

⁸ Patches and intradermal administration are each discussed in the application at page 24, lines 19-27, and in the table on page 25.

⁹ Pharmaceutical dosage forms which when administered to a patient establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum are discussed in the application at page 24, lines 19-27, and in the table on page 25.

¹⁰ Described in the application at, for example, page 4, lines 2-3.

¹¹ Described in the application at, for example, in the application at page 24, lines 19-24, and in the table on page 25.

¹² Described in the application at, for example, page 24, lines 19-22.

¹³ The ability to limit the pharmacological effect of desmopressin to a time between four and six hours and to decrease urine production is discussed in the application at page 32, lines 19-23, and at page 36, lines 5-7.

¹⁴ Pharmaceutical dosage forms for intranasal administration which when administered to a patient establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum are discussed in the application at page 24, lines 19-27, and in the table on page 25..

Claim 29

The invention of claim 29 is directed to a pharmaceutical dosage form comprising desmopressin and a pharmaceutically acceptable carrier¹⁵ for *intradermal or transdermal* administration¹⁶ which when administered to a patient *establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum*¹⁷ for a time between four and six hours and decreases urine production.¹⁸

Claim 30

The invention of claim 30 is directed to a pharmaceutical dosage form according to claim 29 that establishes a steady plasma/serum desmopressin concentration of *from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum*.¹⁹

Claim 31

The invention of claim 31 is directed to a pharmaceutical dosage form according to claim 29 that comprises between 0.05 µg and 10 µg desmopressin.²⁰

Claim 32

The invention of claim 32 is directed to a pharmaceutical dosage form according to claim 29 that is adapted for intradermal administration and comprises a patch.²¹

¹⁵ Described in the application at, for example, page 4, lines 2-3.

¹⁶ Described in the application at, for example, page 24, lines 19-27, and in the table on page 25.

¹⁷ Described in the application at, for example, page 24, lines 19-22.

¹⁸ The ability to limit the pharmacological effect of desmopressin to a time between four and six hours and to decrease urine production is discussed in the application at page 32, lines 19-23, and at page 36, lines 5-7.

¹⁹ Pharmaceutical dosage forms for intradermal or transdermal administration which when administered to a patient establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum are discussed in the application at page 24, lines 19-27, and in the table on page 25.

²⁰ Pharmaceutical dosage forms comprising between 0.05 µg and 10 µg desmopressin are discussed in the application at page 6, lines 20-21.

²¹ Patches and intradermal administration are each discussed in the application at page 24, lines 19-27, and in the table on page 25.

VI. GROUND OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether any of claims 29-30 and 32-33 are anticipated under 35 U.S.C. § 102(b) by U.S. Patent No. 5,135,480 ("Bannon," a copy of which is attached as Exhibit E).

2. Whether any of claims 29-30 and 32-33 are anticipated under 35 U.S.C. § 102(b) by U.S. Patent No. 4,878,892 ("Sibalis," a copy of which is attached as Exhibit F).

3. Whether any of claims 1, 3, 4, 9 and 27-31 are anticipated under 35 U.S.C. § 102(b) by Fjellestad-Paulsen (Doctoral Dissertation), "Absorption and Metabolism of Neurohypophyseal Hormones, with special reference to Desmopressin (dDAVP), in Human Tissue and after Various Routes of Administration," 25 May 1996, ("Fjellestad-Paulsen," a copy of which is attached as Exhibit G).

4. Whether any of claims 1, 3-4, 6-7, 9 and 27-33 are unpatentable under 35 U.S.C. § 103(a) over Fjellestad-Paulsen in view of Sibalis or Bannon.

5. Whether any of claims 1, 3, 4, 6, 7, 9 and 27-33 are properly provisionally rejected for obviousness-type double patenting as unpatentable over claims 19-27 of copending U.S. Application No. 12/173,072.

6. Whether any of claims 1, 3, 4, 6, 7, 9 and 27-33 are properly provisionally rejected for obviousness-type double patenting as unpatentable over claims 19-29 of copending U.S. Application No. 12/173,074, now issued as U.S. Patent No. 7,579,321, a copy of which is attached as Exhibit B.

VII. ARGUMENT

The appealed claims are directed to novel desmopressin pharmaceutical compositions and dosage forms. Prior art desmopressin compositions generated high peak levels of drug in the patient's bloodstream,²² putting patients at risk of severe hyponatraemia: "Certain patients

²² As discussed, for example, in the Examiner's "Reasons for Allowance" in the Notice of Allowance of related U.S. Patent No. 7,405,203 (Exhibit D, pp. 4-5).

taking desmopressin are at risk for developing severe hyponatremia that can result in seizures and death.”²³

The pharmaceutical compositions and dosage forms of the present invention are adapted to establish in a patient a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to a maximum of 10.0 picograms per mL plasma/serum. Such pharmaceutical compositions and dosage forms are effective to decrease urine production.

None of the applied references teaches such a dosage form. None teaches that such a desmopressin dose would be effective. None teaches that such a desmopressin dose would decouple the therapeutic benefits of desmopressin from its side effects.

Indeed, the Office has repeatedly acknowledged that the use of desmopressin to achieve a maximum desmopressin plasma/serum concentration no greater than 10 pg/ml is novel and inventive.²⁴

This week, the present application will have been pending for six years. To date, ***no prior art has been identified that teaches a desmopressin dosage form with the claimed pharmacokinetic profile or medical advantages.*** The applied references:

- do not appreciate that desmopressin can be effective at single digit pg/mL concentrations in the bloodstream;
- do not suggest the desirability of limiting the duration of anti-diuretic activity of desmopressin;
- do not suggest that it is possible to limit the duration of the anti-diuretic activity of desmopressin;
- do not suggest how to limit the duration of the anti-diuretic activity of desmopressin;
- do not suggest how to create a dose form that decouples hyponatremia risk from the anti-diuretic efficacy of desmopressin therapy; and

²³ See, for example, the FDA Notice (Exhibit C, p. 3).

²⁴ See, for example, related U.S. Patent No. 7,405,203 (Exhibit A), the Examiner's Reasons for Allowance (Exhibit D pp. 4-5), and related U.S. Patent No. 7,579,321 (Exhibit B).

- do not suggest that it is possible safely to use desmopressin to treat adult nocturia, or to interrupt urine production safely for specific time intervals.

The observation in the Office action that the discovery of a “property which is inherently present in the prior art does not necessarily make [a] claim patentable” (Office action, p. 2) is *irrelevant*, as the invention is *not directed to a composition existing in the prior art*, inherently or otherwise.

The Office action provides *no basis for concluding that any prior art composition expressly or inherently possesses all features* of the claimed invention. *Instead*, the Office action repeatedly takes the position “that any prior art composition would anticipate, or render obvious, the claimed composition” (Office action, p. 3, emphasis in original). This position is facially inconsistent with applicable law, as only prior art that teaches or suggests “not only all of the limitations claimed but also all of the limitations combined in the same way as recited in the claim”²⁵ could anticipate or render obvious the claimed composition. Perhaps to support his position, the Examiner misstates Appellant’s position as being “that the composition make up is irrelevant.”

It is not, and has never been, Appellant’s position that “the composition make up is irrelevant.” The particular formulation of a dosage form can have substantial effects on its rate of delivery to the bloodstream, as is well known in the art and reconfirmed by the very art relied upon in the rejections. (See, for example, Bannon, as discussed below.) It is true that the claimed invention is not limited to one specific intranasal, transdermal or intradermal dosage form. However, using the teachings in the specification and without the exercise of invention, a person of ordinary skill can prepare any of a variety of dosage forms adapted to achieve the advantages of the claimed invention, as evidenced, for example, by the withdrawal of all rejections under 35 U.S.C. § 112. *Nevertheless*, the “composition make up” is *necessarily* relevant, as the functional limitations of the claims require that the dosage form necessarily must have a *structure and composition sufficient to achieve the recited result*. The claims are limited to those dosage forms having such a structure and composition, even if the claims are not limited to one *particular* structure or composition. The evidence of record provides no basis for

²⁵ *NetMoneyIN, Inc. v. Verisign, Inc.*, 545 F.3d 1359 (Fed. Cir. 2008).

concluding that any prior art desmopressin dosage form has a structure and composition achieving the *novel* and advantageous properties of Appellant's *novel* compositions.

Indeed, although desmopressin formulations have existed for many years, and their resulting rates of accumulation in the blood have been measured for many years, none of the prior art desmopressin formulations of record have ever been reported to achieve the effects of the presently claimed compositions.²⁶ As such, the claimed advantages were clearly *not* inherent in all (or any) prior art compositions, despite the contrary position repeatedly taken in the Office action.

1. The rejection of claims 29-30 and 32-33 as anticipated under 35 U.S.C. § 102(b) by Bannan is improper and should be reversed.

Claims 29 and 33

Bannan does not anticipate either claim 29 or claim 33. Claim 29 (and claim 33, which depends from claim 29) requires a pharmaceutical dosage form which establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum. Bannan discloses transdermal drug delivery devices and discloses desmopressin dissolved in a gel made from karaya gum at a concentration of 3 mg/mL. Bannan does *not* expressly or inherently disclose that a steady desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum would be established.

Bannan even fails to disclose sufficient facts permitting speculation on the plasma/serum levels that its described transdermal product might establish. For example, Bannan does *not* disclose a total amount of desmopressin in a pharmaceutical dosage form, does *not* disclose a rate at which desmopressin would travel within the dosage form, does *not* disclose a rate at which desmopressin in karaya gum would penetrate skin, and does *not* disclose an overall rate of desmopressin delivery to the bloodstream.

²⁶ See, for example, Table 7 at pages 50 and 51 of Fjellestad-Paulsen, in which all of the reported peak desmopressin plasma concentrations (C_{\max}) exceeded 30 pg/mL, exceeding the presently claimed range.

The Office action points to **no evidence** that Bannon expressly or inherently establishes a steady desmopressin concentration within the claimed range. Rather, the Examiner takes the position “that any prior art composition would anticipate the instantly claimed composition and that the requisite function is inherent” (Office action, p. 6, emphasis in original).

The Examiner’s position cannot be reconciled with the facts or the law.

A prior art reference does not anticipate unless it discloses “within the four corners of the document not only all of the limitations claimed but also all of the limitations combined in the same way as recited in the claim.” *NetMoneyIN, Inc. v. Verisign, Inc.*, 545 F.3d 1359 (Fed. Cir. 2008).

To establish that a reference inherently discloses a specific limitation, the Examiner may refer to **extrinsic evidence** showing that the descriptive matter missing from the reference is **necessarily** present in the reference’s disclosure. *Continental Can v. Monsanto Co.*, 948 F.2d 1264, 1268 (Fed. Cir. 1991). Thus, the Examiner cannot establish inherency merely by demonstrating that the asserted limitation is probable or possible. *In re Oelrich*, 666 F.2d 578, 581 (CCPA 1981). “To establish inherency, the extrinsic evidence ‘must make clear that the missing descriptive material is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.’” *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999). “In relying upon the theory of inherency, **the examiner must provide a basis in fact and/or technical reasoning** to reasonably support the determination that the allegedly inherent characteristic **necessarily** flows from the teachings of the applied prior art.” *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990), emphasis added.

This anticipation rejection must be reversed, as the Examiner has provided **no** extrinsic evidence showing that Bannon teaches a dosage form which **necessarily** establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a **maximum** of 10.0 picograms desmopressin per mL plasma/serum.

To the contrary, the extrinsic and intrinsic evidence agree that transdermal delivery is variable. “[T]ransdermal bioavailability is poor because only a small fraction of the drug administered is able to reach a patient’s bloodstream ... through the skin.” *Declaration of Ronald V. Nardi Under 37 C.F.R. § 1.132* (“Nardi Declaration,” a copy of which is attached as Exhibit H), paragraph 10. “Transdermal bioavailability is dependent on many formulation factors known to the skilled artisan.” *Id.* Bannon itself makes clear that the rate of administration (one of the several pieces of information required even to speculate on an established plasma/serum desmopressin concentration) varies depending on the design parameters selected for a particular dosage form. For example, Bannon teaches that:

- administration rates can be enhanced by including “absorption promoters” such as aprotic solvents or surfactants in the dosage form (col. 1, lines 52-55)
- rates of drug release can be varied based on “whether the drug molecules are suspended or dissolved in the vehicle and on the interfacial partition coefficient of the drug between the delivery system and the skin” (col. 1, lines 65-68); and
- rates of release of an active constituent can be controlled “by matrix diffusion or by its passage through a controlling membrane” (col. 2, lines 9-11).

While such parameters could, of course, be modulated when producing a dosage form according to the invention of claim 29, there is no evidence that any dosage form described in Bannon dosage form establishes a steady desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum.

The art of record establishes that art-recognized design parameters will vary the rate of drug administration. As such, the Examiner’s assertion that any prior art composition would anticipate the invention of claim 29, is ***not merely unsupported*** by the evidence of record, but ***cannot be reconciled*** with the teachings of the very reference on which the Examiner relies for the rejection.

Furthermore, claim 29 requires that the pharmaceutical dosage form establish the claimed desmopressin concentration for a time between four and six hours. This valuable feature of the dosage forms of claim 29 restores urine production after a defined interval, reducing the risk of hyponatremia. Bannon does not expressly or inherently disclose a

transdermal dosage form that establishes the claimed desmopressin concentration for a time between four and six hours. The Examiner has provided ***no evidence*** that any dosage form disclosed in Bannon ***necessarily*** establishes the claimed desmopressin concentration for a time ***limited*** to between four and six hours. Bannon therefore cannot anticipate claim 29.

Claim 30

Bannon does not anticipate claim 30 for all of the reasons that it fails to anticipate claim 29. Furthermore, claim 30 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has provided ***no evidence*** that any Bannon composition ***necessarily*** establishes a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 30. Bannon therefore does not anticipate claim 30.

Claim 32

Bannon does not anticipate claim 32 for all of the reasons that it fails to anticipate claim 29. Furthermore, claim 32 requires that the dosage form comprises a patch. Bannon teaches transdermal drug delivery device that comprises an electrode. The Examiner has not argued that Bannon teaches a desmopressin dosage form comprising a patch. For this additional reason, the rejection of claim 32 must be reversed.

Moreover, claim 32 requires that the dosage form be adapted for ***intradermal*** administration. Bannon teaches ***transdermal*** devices (see title). Whereas transdermal delivery involves application of drug to an exterior surface of the skin, such that the drug must cross the stratum corneum before reaching the viable epidermis or dermis,²⁷ ***intradermal*** delivery involves administration of the drug ***within*** the skin (***beyond*** the stratum corneum). The Examiner has not argued that Bannon teaches a dosage form adapted for intradermal administration. For this additional reason, the rejection of claim 32 must be reversed.

²⁷ See, for example, the abstract of Meyer *et al.* (1988), "Successful transdermal administration of therapeutic doses of a polypeptide to normal human volunteers," *Clin. Pharmacol. & Therapeutics* 44(6):607-612 (a copy of which is attached as Exhibit I): "The human stratum corneum constitutes a relatively impermeable barrier to the transdermal absorption of most substances."

2. The rejection of claims 29-30 and 32-33 as anticipated under 35 U.S.C. § 102(b) by Sibalis is improper and should be reversed.

Claims 29 and 33

Sibalis does not anticipate either claim 29 or 33. Claim 29 (and claim 33, which depends from claim 29) requires a pharmaceutical dosage form which establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a **maximum** of 10.0 picograms desmopressin per mL plasma/serum. Sibalis discloses transdermal patches for delivering polypeptides such as desmopressin, but does **not** disclose any particular **amount** of desmopressin, any **rate** at which the desmopressin would be delivered, or a **concentration that would be established** in the plasma or serum of a patient.

The Office action points to **no evidence** that any disclosed Sibalis device can or will expressly or inherently establish a steady desmopressin concentration within the claimed range. Rather, the Examiner takes the inappropriate position “that any prior art composition would anticipate the instantly claimed composition and that the requisite function is inherent” (Office action, p. 5, emphasis in original).

Despite the Examiner’s expressed views, a prior art reference does not anticipate unless it discloses “within the four corners of the document not only all of the limitations claimed but also all of the limitations combined in the same way as recited in the claim.” *NetMoneyIN, Inc. v. Verisign, Inc.*, 545 F.3d 1359 (Fed. Cir. 2008). To establish that a reference inherently discloses a specific limitation, the Examiner may refer to **extrinsic evidence** showing that the descriptive matter missing from the reference is **necessarily present** in the reference’s disclosure. *Continental Can v. Monsanto Co.*, 948 F.2d 1264, 1268 (Fed. Cir. 1991). Thus, the Examiner cannot establish inherency merely by demonstrating that the asserted limitation is probable or possible. *In re Oelrich*, 666 F.2d 578, 581 (CCPA 1981). “To establish inherency, the extrinsic evidence ‘must make clear that the missing descriptive material is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.’” *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999). “In relying upon the theory of inherency, **the**

examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic ***necessarily*** flows from the teachings of the applied prior art.” *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990), emphasis added.

The anticipation rejection must be reversed, as the Examiner has provided ***no extrinsic evidence*** showing that Sibalix teaches a dosage form having characteristics that ***necessarily*** results in establishing a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a ***maximum*** of 10.0 picograms desmopressin per mL plasma/serum.

To the contrary, the extrinsic evidence indicates that bioavailability for transdermal delivery is ***variable***. Transdermal delivery varies based on the specific formulation used, as explained in the *Nardi Declaration* (Exhibit H) at paragraph 10:

- “[T]ransdermal bioavailability is poor because only a small fraction of the drug administered is able to reach a patient’s bloodstream ... through the skin.”
- “Transdermal bioavailability is dependent on many formulation factors known to the skilled artisan.”

Indeed, Bannon, the reference applied in the previously discussed rejection, makes clear that the rate of administration (one of the several pieces of information required even to speculate on an established plasma/serum desmopressin concentration) varies depending on the design parameters selected for a particular dosage form. For example, Bannon teaches that:

- administration rates can be enhanced by including “absorption promoters” such as aprotic solvents or surfactants in the dosage form (col. 1, lines 52-55)
- rates of drug release can be varied based on “whether the drug molecules are suspended or dissolved in the vehicle and on the interfacial partition coefficient of the drug between the delivery system and the skin” (col. 1, lines 65-68); and
- rates of release of an active constituent can be controlled “by matrix diffusion or by its passage through a controlling membrane” (col. 2, lines 9-11).

While such parameters could, of course, be modulated when producing a dosage form according to the invention of claim 29, there is no evidence that the dosage form of Sibalix establishes a steady desmopressin concentration in the range of from about 0.1 picograms

desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum. Sibalis ***does not even disclose an amount of desmopressin*** in the dosage form, ***a desmopressin concentration*** used, or any other information that would be required even to speculate on what desmopressin concentration profile would result in a patient—and certainly does not permit a conclusion that its dosage form ***necessarily*** establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a ***maximum*** of 10.0 picograms desmopressin per mL plasma/serum.

Furthermore, claim 29 requires that the pharmaceutical dosage form must be adapted to establish the claimed desmopressin concentration for a time between four and six hours. It is untrue that any transdermal desmopressin patch will establish the claimed desmopressin concentration for a time between four and six hours, and the Examiner has provided ***no evidence*** that any dosage form described by Sibalis would do so. Sibalis therefore cannot anticipate claim 29 (or dependent claim 32 or 33).

Claim 30

Sibalis does not anticipate claim 30 for all of the reasons that it fails to anticipate claim 29. Furthermore, claim 30 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has provided ***no evidence*** that the Sibalis dosage form ***necessarily*** establishes a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 30. Sibalis therefore cannot anticipate claim 30.

Claim 32

Sibalis does not anticipate claim 32 for all of the reasons that it fails to anticipate claim 29. Furthermore, claim 32 requires that the dosage form be adapted for ***intra***dermal administration. Sibalis relates to “Electrolytic ***Trans***dermal Delivery of Polypeptides” (Title, emphasis added). The Examiner has provided no evidence that the Sibalis discloses a dosage form adapted for ***intra***dermal administration. For this additional reason, the rejection of claim 32 must be reversed.

3. The rejection of claims 1, 3, 4, 9 and 27-31 as anticipated under 35 U.S.C. § 102(b) by Fjellestad-Paulsen is improper and should be reversed.

Claims 1 and 3

As a threshold matter, note that the Office action presents no *arguments* that Fjellestad-Paulsen anticipates any of the appealed claims.

The entirety of the rejection, presented at pages 4 and 5 of the Office action, is a conclusion that the claims are rejected, followed by citations to various desmopressin compositions disclosed in the applied reference. No explanation is provided as to which, if any, of the desmopressin concentrations are asserted to anticipate which, if any, of the appealed claims. As but one example: “Fjellestad-Paulsen additionally teaches sublingual, oral, transdermal, intratracheal, aerosol, rectal and ocular administrations of desmopressin are known in the art” (Office action, p. 4). No connection is offered between any one of these “known in the art” desmopressin administration methods and any of the limitations of any of the appealed claims. What relevance, if any, does a discussion of sublingual administration in Fjellestad-Paulsen have to the method of claim 1? What relevance to the method of claim 3? To reject claims as anticipated, the USPTO bears the burden of proving, by a preponderance of the evidence, that “within the four corners of the document not only all of the limitations claimed but also all of the limitations combined in the same way as recited in the claim.” *NetMoneyIN, Inc. v. Verisign, Inc.*, 545 F.3d 1359 (Fed. Cir. 2008). This, the Office action has not done. Appellant and the Board are left to speculate on the basis for the Examiner’s belief that one or more dosage forms disclosed in Fjellestad-Paulsen anticipate claims 1, 2, 4, 9, and 27-31. The rejection of claims 1, 3, 4, 9 and 27-31 is therefore improper and should be reversed.

In any event, Fjellestad-Paulsen does not disclose any pharmaceutical composition anticipating claim 1 (or 3, which depends from claim 1). Claim 1 requires “a pharmaceutical composition comprising ... desmopressin and a pharmaceutically acceptable carrier in *a dosage form adapted for* intranasal, transdermal, or intradermal *administration sufficient to establish* in a patient *a steady plasma/serum concentration in the range* of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum and to decrease urine production.” Fjellestad-Paulsen does not teach a dosage

form adapted for administration to establish a steady plasma/serum concentration in the claimed range and therefore cannot anticipate claim 1. Anticipation requires that a reference expressly or inherently disclose not only all of the limitations claimed but also all of the limitations arranged or combined in the same way as recited in the claim. *Net MoneyIN, Inc. v. Verisign, Inc.*, 545 F.3d 1359 (Fed. Cir. 2008). As **none** of the pharmaceutical compositions disclosed in Fjellestad-Paulsen **expressly** meets all of the limitations of **any** of the claims, any anticipation would be based, if at all, on the inherency doctrine.

Because Fjellestad-Paulsen discloses no pharmaceutical composition expressly or inherently having all of the limitations of claim 1, Fjellestad-Paulsen cannot anticipate claim 1.

The subcutaneous and intravenous dose forms are not adapted for intradermal administration.

The Office action references subcutaneous doses of desmopressin disclosed at pages 15, 37 and 50 of Fjellestad-Paulsen and an intravenous dose also disclosed at page 50. In contrast, claim 1 requires a dosage form adapted for ***intranasal, transdermal, or intradermal administration***. The Office action asserts that “subcutaneous administration is a form of intradermal administration,” but provided no evidentiary support for the assertion. The assertion is, in fact, clearly erroneous, as “subcutaneous” means “under the skin” and “intradermal” means “within the skin.” As the two terms refer to two distinct, non-overlapping locations (under versus within), even the broadest reasonable interpretation of intradermal is inconsistent with the Examiner’s position.

Perhaps recognizing as tenuous the allegation equating intradermal and subcutaneous administration, the Office action also posits that “nothing precludes intradermally injecting” the doses that include sodium chloride. As a threshold matter, the Examiner has provided no evidence that the subcutaneous or intravenous doses of Fjellestad-Paulsen are ***adapted for intradermal*** (or intranasal or transdermal) administration. The Examiner’s failure to provide such evidence is sufficient grounds for reversing the rejection of claim 1, to the extent the rejection is based on the subcutaneous or intravenous doses.

The subcutaneous and intravenous dose forms are not adapted to establish a plasma/serum concentration in the claimed range.

Furthermore, the Examiner has provided no evidence or argument that the subcutaneous or intravenous doses would establish a steady plasma/serum concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a *maximum* of 10.0 picograms desmopressin per mL plasma/serum. As such, the doses cannot anticipate claim 1.

The law of inherency prohibits a finding of anticipation unless the described prior art composition *necessarily* meets the claim limitations. To establish that a reference inherently discloses a specific limitation, the Examiner may refer to *extrinsic evidence* showing that the descriptive matter missing from the reference is *necessarily* present in the reference's disclosure. *Continental Can v. Monsanto Co.*, 948 F.2d 1264, 1268 (Fed. Cir. 1991).

The anticipation rejection must be reversed, as the Examiner has provided *no reasoning* and *no extrinsic evidence* showing that Fjellestad-Paulsen teaches a dosage form that necessarily establishes a steady plasma/serum desmopressin concentration within the claimed range.

Indeed, the very reference relied upon by the Examiner makes clear that the *bioavailability of a peptide hormone will vary substantially based on the route of administration, the type of delivery system, and the formulation*. Fjellestad-Paulsen, pp. 16-17. For example, a 2 µg subcutaneous or intravenous dose, even if somehow administered intradermally, would be essentially 100% bioavailable, leading to plasma/serum concentrations well in excess of the claimed range.

As the Examiner has failed to show that the subcutaneous and intravenous Fjellestad-Paulsen dosage forms are *adapted for* intranasal, transdermal, or *intra dermal administration sufficient to establish* in a patient *a steady plasma/serum concentration in the range* of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum, the rejection of claim 1 as anticipated by those dosage forms must be reversed.

The intranasal dose forms are not adapted to establish a plasma/serum concentration in the claimed range.

The Office action also references various intranasal doses disclosed at page 14 of Fjellestad-Paulsen.

Again, the Examiner has provided ***no argument*** and ***no evidence*** that any dose described in Fjellestad-Paulsen would establish a steady plasma/serum concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum. As such, the doses cannot anticipate claim 1. Unlike intravenous or intradermal delivery routes, providing a predictably high bioavailability, the bioavailability of peptide hormones such as desmopressin is ***highly variable when administered intranasally, depending on the delivery mechanism and formulation***. See, e.g., Fjellestad-Paulsen, p.16:

- “Depending on various factors, such as ... the type of delivery system and the galenic formulation, the bioavailability of 10-20 amino acid peptides in general, given intranasally, varies between 1 and 12%.”
- “In one of the earliest studies on dDAVP [desmopressin] the bioavailability of i.n. [intranasal] dDAVP in [diabetes insipidus] patients was estimated to be 10-20%.”
- “In [a more recent] study the bioavailability was 10% after i.n. administration.”
- “Köhler and Harris (1998) ... found a bioavailability as low as 2% after i.n. administration.”

Appellant agrees that it is ***possible*** to prepare dosage forms adapted for intranasal delivery that establish a steady plasma/serum concentration within the claimed range. Before the present invention, however, there was no reason to do so. More to the point, there is no reason to believe that any dosage form disclosed in Fjellestad-Paulsen would have intentionally or accidentally established a steady plasma/serum concentration within the claimed range, as prior art formulations intended to establish much higher concentrations. Prior art desmopressin dosage forms were designed to achieve higher desmopressin concentrations in the blood, as

acknowledged in the issuance of related U.S. patents.²⁸ Indeed, for those experiments *where Fjellestad-Paulsen does report a peak desmopressin concentration* in, Table 7 at pages 50 and 51, *all of the reported peak desmopressin plasma concentrations (C_{max}) exceeded 30 pg/ml, exceeding the presently claimed range.*

Because the compositions of claim 1 are adapted to establish a steady plasma/serum desmopressin concentration within the claimed range, and because the Examiner has provided *no evidence* that any composition described by Fjellestad-Paulsen *necessarily* establishes a steady plasma/serum desmopressin concentration within the claimed range, the rejection of claim 1 as anticipated by Fjellestad-Paulsen must be reversed.

Claim 4

Fjellestad-Paulsen does not anticipate claim 4 for the reasons that it fails to anticipate claim 1 or 3. Furthermore, claim 4 requires that the pharmaceutical composition comprise from about 0.1 µg to about 2 µg desmopressin. Fjellestad-Paulsen references subcutaneous or intravenous doses of 2 µg desmopressin; these would generate a peak desmopressin plasma/serum concentration exceeding the claimed range. At page 14, Fjellestad-Paulsen also references desmopressin doses for infants:

The most prevalent route of administration is the intranasal (i.n.). Children and adult patients usually require 5-20 µg dDAVP intranasally once or twice daily ... and infants are treated with smaller doses ranging from 1 to 15 µg once or twice a day.

Fjellestad-Paulsen specifies that the doses for children and adults is intranasal; the route of administration to infants is not specified. More importantly, Fjellestad-Paulsen provides *no information about the formulation* or other characteristics of any dosage form for an infant that would permit a conclusion that it was adapted to establish a plasma/serum desmopressin concentration in the claimed range. The Examiner has provided *no evidence* that any pharmaceutical composition disclosed in Fjellestad-Paulsen and comprising about 0.1 µg to about 2 µg desmopressin would *necessarily* establish a steady plasma/serum concentration range

²⁸ See, for example, related U.S. Patent No. 7,405,203 (Exhibit A), the Examiner's Reasons for Allowance (Exhibit D, p. 4), and related U.S. Patent No. 7,579,321 (Exhibit B).

recited in claim 1. For this additional reason, the rejection of claim 4 as anticipated by Fjellestad-Paulsen must be reversed.

Claim 9

Fjellestad-Paulsen does not anticipate claim 9 for the reasons that it fails to anticipate claim 1 or 3. Furthermore, claim 9 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has provided **no evidence** that any composition described in Fjellestad-Paulsen **necessarily** establishes a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 9. For this additional reason, the rejection of claim 9 as anticipated by Fjellestad-Paulsen must be reversed.

Claim 27

Fjellestad-Paulsen does not anticipate claim 27 for the reasons that it fails to anticipate claim 1 or 3. Furthermore, claim 27 requires a dosage form adapted for intranasal administration, obviating any issues relating to the intravenous or subcutaneous doses of Fjellestad-Paulsen, and requires that the desmopressin plasma/serum concentration be established for a time between four and six hours. The Examiner has provided **no evidence** that the Fjellestad-Paulsen intranasal compositions **necessarily** establish a steady plasma/serum desmopressin concentration within the claimed range for a time between four and six hours. For this additional reason, the rejection of claim 27 as anticipated by Fjellestad-Paulsen must be reversed.

Claim 28

Fjellestad-Paulsen does not anticipate claim 28 for all of the reasons that it fails to anticipate claim 27. Furthermore, claim 28 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has provided **no evidence** that the Fjellestad-Paulsen compositions **necessarily** establish a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 28. For

this additional reason, the rejection of claim 28 as anticipated by Fjellestad-Paulsen must be reversed.

Claims 29 and 31

Fjellestad-Paulsen does not anticipate claim 29 (or claim 31, which depends from claim 29) for the reasons that it fails to anticipate claim 1 or 3. Furthermore, claim 29 requires a dosage form adapted for intradermal or transdermal administration, obviating any issues relating to the intranasal doses of Fjellestad-Paulsen, and requires that the desmopressin plasma/serum concentration be maintained within the recited low dose range for a time between four and six hours. The Examiner has provided ***no evidence*** that the Fjellestad-Paulsen compositions ***necessarily*** establish a steady plasma/serum desmopressin concentration within the claimed range for a time between four and six hours. For this additional reason, the rejection of claims 29 and 31 as anticipated by Fjellestad-Paulsen must be reversed.

Claim 30

Fjellestad-Paulsen does not anticipate claim 30 for all of the reasons that it fails to anticipate claim 29. Furthermore, claim 30 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has provided ***no evidence*** that any composition described by Fjellestad-Paulsen composition ***necessarily*** establishes a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 30. For this additional reason, the rejection of claim 30 as anticipated by Fjellestad-Paulsen must be reversed.

4. The rejection of claims 1, 3-4, 6-7, 9 and 27-33 as unpatentable under 35 U.S.C. § 103(a) over Fjellestad-Paulsen in view of Sibalís or Bannon is improper and should be reversed.

The question of obviousness, of course, is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the prior art and the subject matter claimed, taken as a whole; and (4) secondary considerations of nonobviousness, if any. *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966). A claim “composed of several elements is not proved obvious merely by

demonstrating that each of its elements was, independently, known in the prior art.” *KSR Int’l v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007). The relevant question is “whether there was an apparent reason to combine the known elements in the fashion claimed by the” patent application. *Id.* “We must still be careful not to allow hindsight reconstruction of references to reach the claimed invention without any explanation as to how or why the references would be combined to produce the claimed invention.” *Innogenetics, N.V. v. Abbott Labs.*, 512 F.3d 1363, 1374 n.3 (Fed. Cir. 2008).

The presently claimed pharmaceutical compositions and dosage forms have important advantages over conventional desmopressin dosage forms, advantages neither taught nor suggested in the prior art. For example, the Office has acknowledged that keeping the peak desmopressin concentration in the bloodstream at or below 10.0 pg/mL was not previously taught or suggested.²⁹ The claimed invention successfully addresses the safety concerns with conventional desmopressin administration. The applied references ***do not discuss or acknowledge*** the safety concerns, much less ***suggest that a solution is possible***. As such, they of course do not suggest that ***the solution could involve reducing*** the peak desmopressin concentration, perhaps because they did not recognize that ***the resulting desmopressin dosage forms would retain their therapeutic efficacy***.

Appellant ***did*** recognize that the resulting desmopressin dosage forms would be safe and effective. Indeed, their safety and efficacy have now propelled them into clinical trials, including a phase III clinical trial that is currently ongoing, providing the promise of safer antidiuresis to patients who would otherwise be at serious risk. The pharmaceutical compositions and dosage forms invented by Appellant to decouple desmopressin’s therapeutic efficacy from the substantial risks of prior art desmopressin dosage forms are nowhere taught or suggested by the applied references.

Claims 1 and 3

Fjellestad-Paulsen in view of Sibalis or Bannon does not render obvious the subject matter of claim 1 (or 3, which depends from claim 1). Claim 1 requires “a pharmaceutical composition comprising ... desmopressin and a pharmaceutically acceptable carrier in a dosage

form ***adapted for*** intranasal, transdermal, or intradermal ***administration sufficient to establish*** in a patient ***a steady plasma/serum concentration in the range*** of from about 0.1 picograms desmopressin per mL plasma/serum to about a ***maximum*** of 10.0 picograms desmopressin per mL plasma/serum and to decrease urine production.”

None of the three cited references teach a dosage form adapted for administration to establish a steady plasma/serum concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum and to decrease urine production.

None of the three cited references teach that it is desirable to, or that there are any advantages in, formulating a desmopressin dosage form to establish a steady plasma/serum concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum and to decrease urine production. Assessment of the presented claims requires that the unobvious properties of the claimed composition be taken into account. Pharmaceutical compositions that establish a blood desmopressin concentration within the claimed range provide the antidiuretic effects of desmopressin in a safe and effective manner:

- Appellant discovered that desmopressin can be effective at single digit pg/mL concentrations in the bloodstream. This was not taught or suggested in the applied references.
- Appellant discovered that desmopressin dosage forms that establish a desmopressin concentration that does not exceed a maximum of 10.0 pg/mL can be used to limit the duration of anti-diuretic activity of desmopressin. The applied references do not suggest that limiting the duration is ***desirable***, do not suggest that it is ***possible***, and do not suggest ***how it would be achieved***. The claimed pharmaceutical compositions decouple desmopressin’s benefits from its most serious risks, and ***neither the objective nor the means is taught or suggested*** by the applied references.

²⁹ Exhibit D, pp. 4-5.

The Examiner has proffered no argument why one of ordinary skill in the art would have attempted to prepare a desmopressin dosage form adapted for administration sufficient to establish a steady plasma/serum concentration in the range claimed in claim 1. The Examiner has argued a “need for smaller doses for smaller patients” (Office action, p. 7), but this was in the context of “dose size” (Office action, p. 6). The Examiner has not attempted to argue that one of ordinary skill had a motivation, prior to the instant invention, to prepare a dosage form adapted for administration to establish a steady plasma/serum concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum and to decrease urine production. Such a dosage form has distinct advantages disclosed in the present application but unappreciated in the art prior to Appellant’s invention.³⁰

“[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness.” *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006). “[T]his analysis should be made explicit.” *KSR International Co., v. Teleflex Inc.*, 550 U.S. 398, ___, 127 S. Ct. 1727, 1741 (2007).

The claimed invention is an important improvement over prior desmopressin compositions. Conventional desmopressin formulations carry a “risk for developing severe hyponatremia that can result in seizures and death.” FDA Notice, Exhibit C. The claimed invention decouples desmopressin’s dangerous side effects from its pharmaceutical benefits: “The dosage forms possessing the features recited in the claims achieve a novel and surprising effect, as they can effectively interrupt urine production – that is, induce voiding postponement, less frequent urination, and other antidiuretic effects, yet avoid, decrease or eliminate induction of hyponatremia. This is accomplished by controlling the duration of the anti-diuretic effect of desmopressin by controlling its blood concentration and switching it ‘off’ at the desired time as the concentration of the circulating drug is cleared by the body and falls below a concentration effective to activate kidney water channels.” *Nardi Declaration*, paragraph 7.

³⁰ Indeed, the advantageous properties of these dosage forms have repeatedly been recognized as patentable, as the USPTO has issued two related U.S. patents (U.S. Patent Nos. 7,405,203 and 7,579,321) to methods of using low dose desmopressin.

As no reasoning has been offered to explain why a person would have modified the dosage forms of Fjellestad-Paulsen, Sibalís or Bannon to establish a desmopressin plasma/serum concentration within the claimed range, achieving the important advantages of the claimed invention, the rejection of claims 1 and 3 under 35 U.S.C. § 103(a) must be reversed.

Claim 4

The three applied references do not render obvious the subject matter of claim 4 for the reasons that they fail to render obvious the subject matter of claim 1 or 3. Furthermore, claim 4 requires that the pharmaceutical composition comprise from about 0.1 µg to about 2 µg desmopressin.

Fjellestad-Paulsen references subcutaneous or intravenous doses of 2 µg desmopressin, which would generate a peak desmopressin plasma/serum concentration exceeding the claimed range. Fjellestad-Paulsen also references 1 to 15 µg desmopressin administration to children, in a dose of unspecified form or formulation. The Examiner has offered no reasoning why a person would have modified any pharmaceutical formulation described in Fjellestad-Paulsen to establish a desmopressin plasma/serum concentration within the claimed range. For this additional reason, the rejection of claim 4 under 35 U.S.C. § 103(a) must be reversed.

Claim 6

The applied references do not render obvious the subject matter of claim 6 for the reasons that they fail to render obvious the subject matter of claim 1 or 3. Furthermore, claim 6 requires that the dosage form be adapted for transdermal delivery. None of the three cited references teaches a dosage form adapted for transdermal delivery that comprises 0.5 ng to 20 µg of desmopressin, nor provides any reason for creating a dosage form adapted for transdermal delivery that comprises 0.5 ng to 20 µg of desmopressin. The Office action acknowledges that none of the references “specifically enumerates the claimed quantity (0.5 ng to 20 µg) in the composition” (Office action, p. 6).

The Office action nevertheless argues that “Sibalís does not provide any specific quantity, and thus embraces all quantities from one molecule of desmopressin to the saturation limit of the composition formed” (Office action, p. 7).

The question, of course, is not what Sibalís *embraces*, but what it specifically discloses or suggests. The Office action does not argue that Sibalís teaches or suggests “any specific quantity”; Sibalís therefore *cannot* suggest the quantity required by claim 6 (as dependent from claim 1).

The Office action also argues that Bannon “overlaps with the instantly claimed range of desmopressin in the composition” (Office action, pp. 6-7). The Office action appears to base this argument on the general teaching in Bannon that a drug substance can be at a concentration of 0.1 to 15% weight/volume (claim 16) and/or the concentration of desmopressin of 3 mg/ml recited in Example 5.

A concentration alone provides no information about the total amount of desmopressin. To know the total amount, one must know the concentration *and* the volume. The Office action has provided no rational basis for its conclusion that Bannon “overlaps with the instantly claimed range of desmopressin.”

The Office action also argues that “[d]ose size is an extrinsic property that does not materially alter the composition” (Office action, p. 6). Appellant disagrees: dose size is a limitation of the claim that cannot be disregarded, and the Office action is not at liberty to disregard it.

The Office action also argues that “one would recognize the need for smaller dosages for smaller patients.”

Smaller than what?

The Office action acknowledged that Sibalís doesn’t disclose a dosage. Bannon discloses a concentration, but not a dosage. Fjellestad-Paulsen discloses no dosage in the context of a dosage form adapted for transdermal delivery.

The Office action has articulated no reason why a person of ordinary skill would have prepared a dosage form adapted for transdermal delivery and comprising 0.5 ng to 20 µg desmopressin, prior to the present invention. Rather, the Examiner argues that, in his view, one would have had a reasonable expectation of success in producing the claimed invention. The Examiner’s position is an insufficient basis for a conclusion of obviousness, both because:

1) an obviousness rejection cannot be sustained in the absence of an articulated motivation for specifically producing a pharmaceutical composition falling within the scope of the claimed invention; and

2) the Examiner has not supported his position that one would have expected the invention of claim 6 to be effective to decrease urine production, as required by the language of claim 1. The appreciation that desmopressin dosage forms comprising 0.5 ng to 20 µg desmopressin and *adapted to establish* a desmopressin plasma/serum concentration within the claimed range would be particularly useful to reduce urine production and would avoid the toxicities observed in the prior art was the *inventor's* appreciation, and is not believed to be found in the prior art. In the absence of evidence relied upon by the Examiner evidencing a specific reason for producing the claimed pharmaceutical composition and a reasonable expectation that it would achieve the claimed benefits, for this additional reason, the rejection of claim 6 under 35 U.S.C. § 103(a) cannot be sustained.

Claim 7

The applied references do not render obvious the subject matter of claim 7 for the reasons that they fail to render obvious the subject matter of claim 1 or 3. Furthermore, claim 7 requires that the pharmaceutical composition be adapted for intradermal administration. None of the applied references teaches a dosage form adapted for intradermal administration. The Office action has articulated no reason why one would have modified the dosage forms of the three cited references to adapt them for intradermal administration. For this additional reason, the rejection of claim 7 under 35 U.S.C. § 103(a) must be reversed.

Claim 9

The applied references do not render obvious the subject matter of claim 9 for the reasons that they fail to render obvious the subject matter of claim 1 or 3. Furthermore, claim 9 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has articulated no reason why one would have modified the dosage forms of the applied references to establish a concentration in the

range claimed in claim 9. For this additional reason, the rejection of claim 9 under 35 U.S.C. § 103(a) must be reversed.

Claim 27

The applied references do not render obvious the subject matter of claim 27 for the reasons that they fail to render obvious the subject matter of claim 1 or 3. Furthermore, claim 27 requires a dosage form adapted for intranasal administration, obviating any issues relating to the intravenous or subcutaneous doses of Fjellestad-Paulsen or to the transdermal devices of Sibalis and Bannon, and requires that the desmopressin plasma/serum concentration be established for a time between four and six hours.

The Examiner has articulated no reason why one would have modified the Fjellestad-Paulsen intranasal compositions to establish a steady plasma/serum desmopressin concentration within the claimed range for a time between four and six hours. Furthermore, no reference known to Appellant taught that limiting the effect of desmopressin was desirable or even possible, much less how to accomplish this while maintaining therapeutic efficacy. For this additional reason, the rejection of claim 27 under 35 U.S.C. § 103(a) must be reversed.

Claim 28

The applied references do not render obvious the subject matter of claim 28 for the reasons that they fail to render obvious the subject matter of claim 27. Furthermore, claim 28 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum.

The Examiner has articulated no reason why one would have modified the Fjellestad-Paulsen intranasal compositions to establish a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 28. For this additional reason, the rejection of claim 28 under 35 U.S.C. § 103(a) must be reversed.

Claims 29 and 33

The applied references do not render obvious the subject matter of claim 29 (or 33, which depends from claim 29) for the reasons that they fail to render obvious the subject matter of claim 1. Furthermore, claim 29 requires a dosage form adapted for intradermal or transdermal administration, obviating any issues relating to the intranasal doses of Fjellestad-Paulsen, and requires that the desmopressin plasma/serum concentration be established for a time between four and six hours. The Examiner has articulated no reason why the prior art transdermal compositions would have been modified to establish a steady plasma/serum desmopressin concentration within the claimed range for a time between four and six hours. Furthermore, no applied reference taught that limiting the effect of desmopressin was desirable or even possible, much less how to accomplish this while maintaining therapeutic efficacy. For this additional reason, the rejection of claims 29 and 33 under 35 U.S.C. § 103(a) must be reversed.

Claim 30

The applied references do not render obvious the subject matter of claim 30 for all of the reasons that they fail to render obvious the subject matter of claim 29. Furthermore, claim 30 requires that the dosage form establish a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum. The Examiner has articulated no reason why the prior art transdermal compositions would have been modified to establish a steady plasma/serum desmopressin concentration within the narrower range claimed in claim 30. For this additional reason, the rejection of claim 30 under 35 U.S.C. § 103(a) must be reversed.

Claim 31

The applied references do not render obvious the subject matter of claim 31 for all of the reasons that they fail to render obvious the subject matter of claim 29. Furthermore, claim 31 requires that the dosage form comprise between 0.05 µg and 10 µg desmopressin. The Examiner has articulated no reason why the prior art transdermal compositions would have been modified to comprise between 0.05 µg and 10 µg desmopressin. Although the Examiner did posit that a Fjellestad-Paulsen desmopressin dose comprising NaCl could be administered intradermally, the Examiner provided no evidentiary support for the proposition and,

importantly, provided no evidence or argument that the dose would establish the desmopressin concentration in the range required by claims 29 and 31. For this additional reason, the rejection of claim 31 under 35 U.S.C. § 103(a) must be reversed.

Claim 32

The three cited references do not render obvious the subject matter of claim 32 for all of the reasons that they fail to render obvious the subject matter of claim 29. Furthermore, claim 32 requires that the dosage form be adapted for intradermal administration and comprise a patch. None of the cited references teaches or suggests a dosage form adapted for intradermal administration and comprising a patch. The Examiner has articulated no reason why the prior art compositions would have been modified to be adapted for intradermal administration and comprising a patch. For this additional reason, the rejection of claim 32 under 35 U.S.C. § 103(a) must be reversed.

5. The provisional rejection of claims 1, 3-4, 6-7, 9 and 27-33 for obviousness-type double patenting as unpatentable over claims 19-27 of copending U.S. Application No. 12/173,072 is improper and should be reversed.

The provisional rejection over claims 19-27 of copending U.S. Application No. 12/173,072 is improper and should be reversed.

The Office action argues that “the compositions used in the ***method of the ‘072*** anticipate the instant claimed compositions” (p. 8, emphasis added).

Claims 19-27 of 12/173,072 are *composition* claims, not *method* claims. No method claims are or were pending in 12/173,072 on January 8, 2009, the date on which the final rejection issued in the present application.

Although Appellant would be prepared to consider filing any necessary terminal disclaimer over, or in, 12/173,012, the reasoning provided in the Office action fails to support the provisional rejection; the rejection must therefore be reversed. Appellant also believes the provisional rejection should be withdrawn in accordance with MPEP § 804(I)(B)(1), as the present application is otherwise allowable.

6. The provisional rejection of claims 1, 3-4, 6-7, 9 and 27-33 for obviousness-type double patenting as unpatentable over claims 19-29 of copending U.S. Application No. 12/173,074 is improper and should be reversed.

The provisional rejection over claims 19-29 of copending U.S. Application No. 12/173,074 is improper and should be reversed. Appellant notes that U.S. Application No. 12/173,074 has now issued as U.S. Patent No. 7,579,321 and that this rejection is presumably no longer “provisional” in nature.

The Office action argues that “the articles of manufacture of ‘074 anticipate the instant claimed compositions being desmopressin compositions of 50 ng to 10 µg (e.g. claim 20), where the composition is for transmucosal, transdermal, intradermal, subcutaneous administration (e.g. claims 22-25).

As a threshold matter, claim 20 of the application did not recite a range of desmopressin concentrations. In fact, no claim of the issued patent recites the range of 50 ng to 10 µg allegedly forming the basis of the obviousness-type double patenting rejection. Moreover, none of claims 22-25 recited that the composition was for transmucosal, transdermal, intradermal, or subcutaneous administration but, rather, were silent regarding routes of administration.

As such, the reasoning provided in the Office action fails to support the provisional rejection, which must be reversed.

Furthermore, the Office action is in error when it asserts that the ‘072 application “is a continuation of the instant application” (Office action, p. 9).

U.S. Application No. 12/173,074, now issued U.S. Patent No. 7,579,321, is a continuation of U.S. Application No. **11/744,615, now U.S. Patent No. 7,405,203, which is a division** of the present application. See Exhibits A and B. The present application was restricted to composition claims, pursuant to a restriction requirement dated April 24, 2006, as reproduced below:

Restriction to one of the following inventions is required under 35 U.S.C. 121:

- I. Claims 1, 3-7, 9, and 14, drawn to a pharmaceutical composition comprising 0.5 mg to 20 ug desmopressin and a pharmaceutical carrier, as well as an article of manufacture whereby the pharmaceutical composition is contained within a packaging material (e.g., a kit), classified in class 435, subclass 810, for example.
- II. Claims 15, 16, and 19-22, drawn to a method of treating or preventing a disease or condition treatable/preventable by desmopressin, classified in class 514, subclass 2, for example.
- III. Claims 17, 18, and 23-26, drawn to a method of inducing an antidiuretic effect in a patient, classified in class 514, subclass 17, for example. The inventions are distinct, each from the other because of the following reasons:

On May 19, 2006, Appellant responded without traverse, electing to prosecute claims drawn to pharmaceutical compositions. The restriction requirement was therefore maintained in the Office action of August 2, 2006. All claims pending in the present application are drawn to compositions; none are drawn to methods. In contrast, U.S. Patent No. 7,405,203 contains only claims drawn to the methods restricted out of the present application; none of the issued claims are drawn to compositions. The claims of U.S. Patent No. 7,579,321 are similarly limited to methods restricted out of the present application; none of its claims are drawn to compositions.

As provided by 35 U.S.C. § 121:

A patent issuing on an application with respect to which a requirement for restriction under this section has been made, ***or on an application filed as a result of such a requirement, shall not be used as a reference*** either in the Patent and Trademark Office or in the courts against a divisional application or ***against the original application*** ... if the divisional application is filed before the issuance of the patent on the other application. (emphasis added)

Because the divisional application leading to the issuance of U.S. Patent No. 7,405,203 and its continuation, U.S. Patent No. 7,579,321, were each filed during the pendency of the present application, and because the claims of each issued patent and its continuation are directed exclusively to subject matter restricted from the present application, neither issued patent can be used as a reference against the present application.

For this reason, too, the double patenting rejection must be reversed.

Dated: November 9, 2009

Respectfully submitted,

/Brian A. Fairchild/

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CLAIMS APPENDIX

1. A pharmaceutical composition comprising 0.5 ng to 20 µg desmopressin and a pharmaceutically acceptable carrier in a dosage form adapted for intranasal, transdermal, or intradermal administration sufficient to establish in a patient a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum and to decrease urine production.
3. The pharmaceutical composition of claim 1 comprising from about 0.05 µg to about 10 µg desmopressin.
4. The pharmaceutical composition of claim 1 comprising from about 0.1 µg to about 2 µg desmopressin.
6. The pharmaceutical composition of claim 1 in a dosage form adapted for transdermal delivery and comprising a patch, gel, cream, ointment, or iontophore.
7. The pharmaceutical composition of claim 1 adapted for intradermal administration comprising a patch.
9. The pharmaceutical composition of claim 1 in a dosage form sufficient to establishes in a patient a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum.
27. A pharmaceutical dosage form comprising desmopressin and a pharmaceutically acceptable carrier adapted for intranasal administration which when administered to a patient establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum for a time between four and six hours and decreases urine production.
28. The composition of claim 27 which establishes a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum.

29. A pharmaceutical dosage form comprising desmopressin and a pharmaceutically acceptable carrier for intradermal or transdermal administration which when administered to a patient establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about a maximum of 10.0 picograms desmopressin per mL plasma/serum for a time between four and six hours and decreases urine production.
30. The dosage form of claim 29 which establishes a steady plasma/serum desmopressin concentration of from about 0.5 picograms desmopressin per mL plasma/serum to about 5.0 picograms desmopressin per mL plasma/serum.
31. The dosage form of claim 29 comprising between 0.05 μg and 10 μg desmopressin.
32. The dosage form of claim 29 adapted for intradermal administration comprising a patch.
33. The dosage form of claim 29 adapted for transdermal delivery and comprising a patch, gel, cream, ointment, or iontophore.

EVIDENCE APPENDIX

| | |
|-----------|---|
| Exhibit A | U.S. Patent No. 7,405,203, which was made of record as reference A73 in the supplemental Information Disclosure Statement of August 15, 2008, which was considered by the Examiner on January 8, 2009. |
| Exhibit B | U.S. Patent No. 7,579,321, which issued from U.S. Application No. 12/173,074, relied upon in the Office action of January 8, 2009. |
| Exhibit C | FDA Notice: Information for Healthcare Professionals Desmopressin Acetate (marketed as DDAVP Nasal Spray, DDAVP Rhinal Tube, DDAVP, DDVP, Minirin, and Stimate Nasal Spray, which was made of record as reference C14 in the supplemental Information Disclosure Statement of August 15, 2008, which was considered by the Examiner on January 8, 2009. |
| Exhibit D | Notice of Allowance, U.S. Patent No. 7,405,203, which was made of record on page 2 of the Information Disclosure Statement letter filed August 15, 2008. |
| Exhibit E | U.S. Patent No. 5,135,480 ("Bannon"), which was made of record as reference A36 in the supplemental Information Disclosure Statement of August 15, 2008, which was considered by the Examiner on January 8, 2009, and relied upon in the Office action of the same date. |
| Exhibit F | U.S. Patent No. 4,878,892 ("Sibalis"), which was made of record as reference A34 in the supplemental Information Disclosure Statement of August 15, 2008, which was considered by the Examiner on January 8, 2009, and relied upon in the Office action of the same date. |
| Exhibit G | Fjellestad-Paulsen (Doctoral Dissertation), "Absorption and Metabolism of Neurohypophyseal Hormones, with special reference to Desmopressin (dDAVP), in Human Tissue and after Various Routes of Administration," 25 May 1996, ("Fjellestad-Paulsen"), which was filed on August 15, 2008, in response to the Request for Information dated July 26, 2008, and was relied upon in the Office action of January 8, 2009. |
| Exhibit H | Declaration of Ronald V. Nardi Under 37 C.F.R. § 1.132, dated May 11, 2007 ("Nardi Declaration"), which was filed with a Request for Continued Examination on May 16, 2007, and was acknowledged at page 2 of the Office action dated October 16, 2007. |
| Exhibit I | Meyer <i>et al.</i> (1988), "Successful transdermal administration of therapeutic doses of a polypeptide to normal human volunteers," <u>Clin. Pharmacol. & Therapeutics</u> 44(6):607-612, which was made of record as reference C5 in the supplemental Information Disclosure Statement of August 15, 2008, which was considered by the Examiner on January 8, 2009. |



US007405203B2

(12) **United States Patent**
Fein

(10) **Patent No.:** **US 7,405,203 B2**
(45) **Date of Patent:** **Jul. 29, 2008**

(54) **PHARMACEUTICAL COMPOSITIONS
INCLUDING LOW DOSAGES OF
DESMOPRESSIN**

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(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **11/744,615**

(22) Filed: **May 4, 2007**

(65) **Prior Publication Data**

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Related U.S. Application Data

(62) Division of application No. 10/706,100, filed on Nov.
12, 2003.

(51) **Int. Cl.**

A61K 38/08 (2006.01)

C07K 7/04 (2006.01)

(52) **U.S. Cl.** **514/16; 530/326**

(58) **Field of Classification Search** None
See application file for complete search history.

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Primary Examiner—Andrew D Kosar

(74) *Attorney, Agent, or Firm*—Goodwin Procter LLP

(57) **ABSTRACT**

The present invention is directed to a pharmaceutical composition comprising 0.5 ng to 20 µg desmopressin and a pharmaceutically acceptable carrier. The present invention is also directed to a pharmaceutical composition comprising desmopressin and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is effective to establish a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about 10.0 picogram desmopressin per mL plasma/serum. Articles of manufacture and methods of using the above invention are also disclosed.

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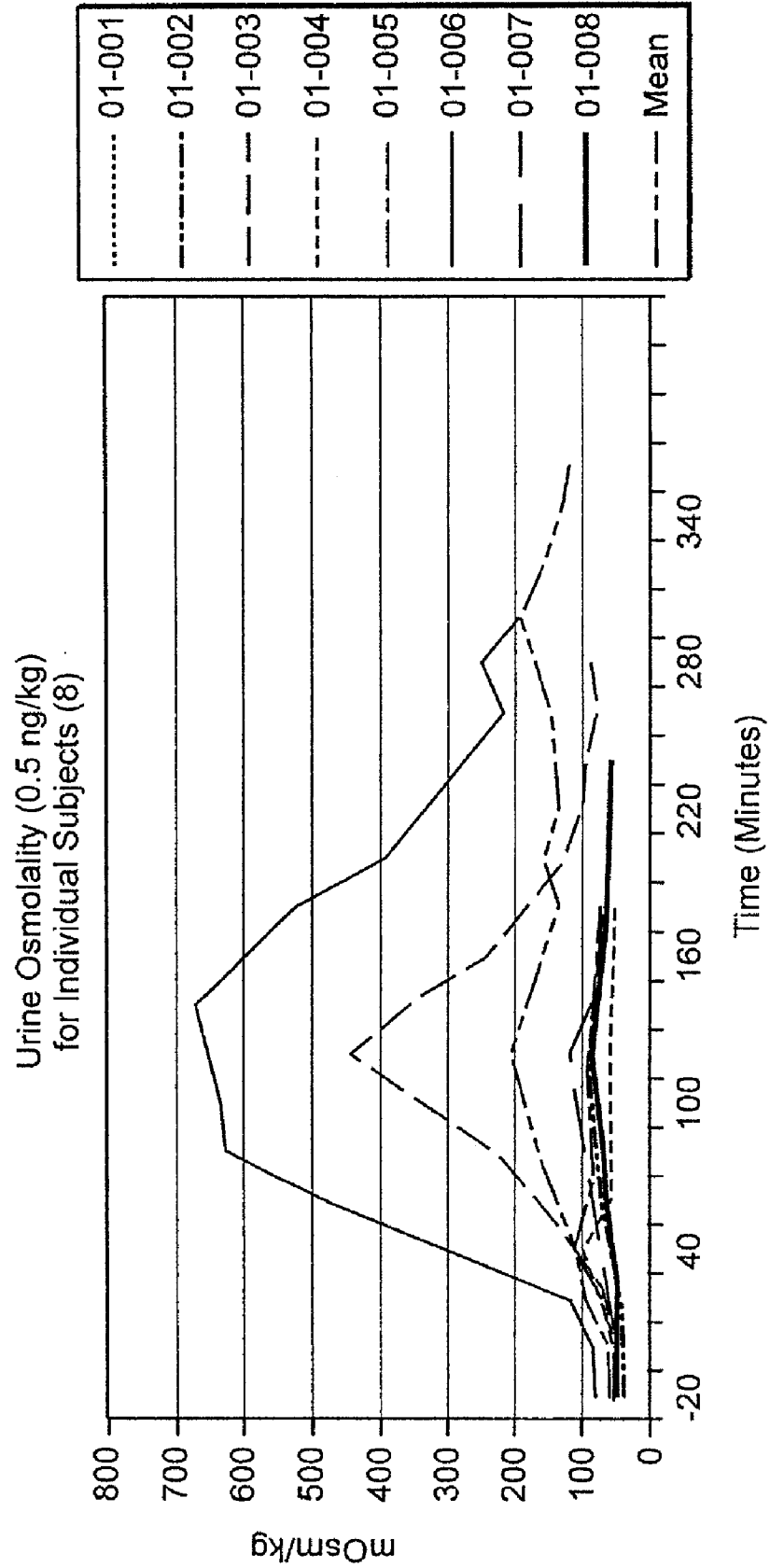


FIG. 1

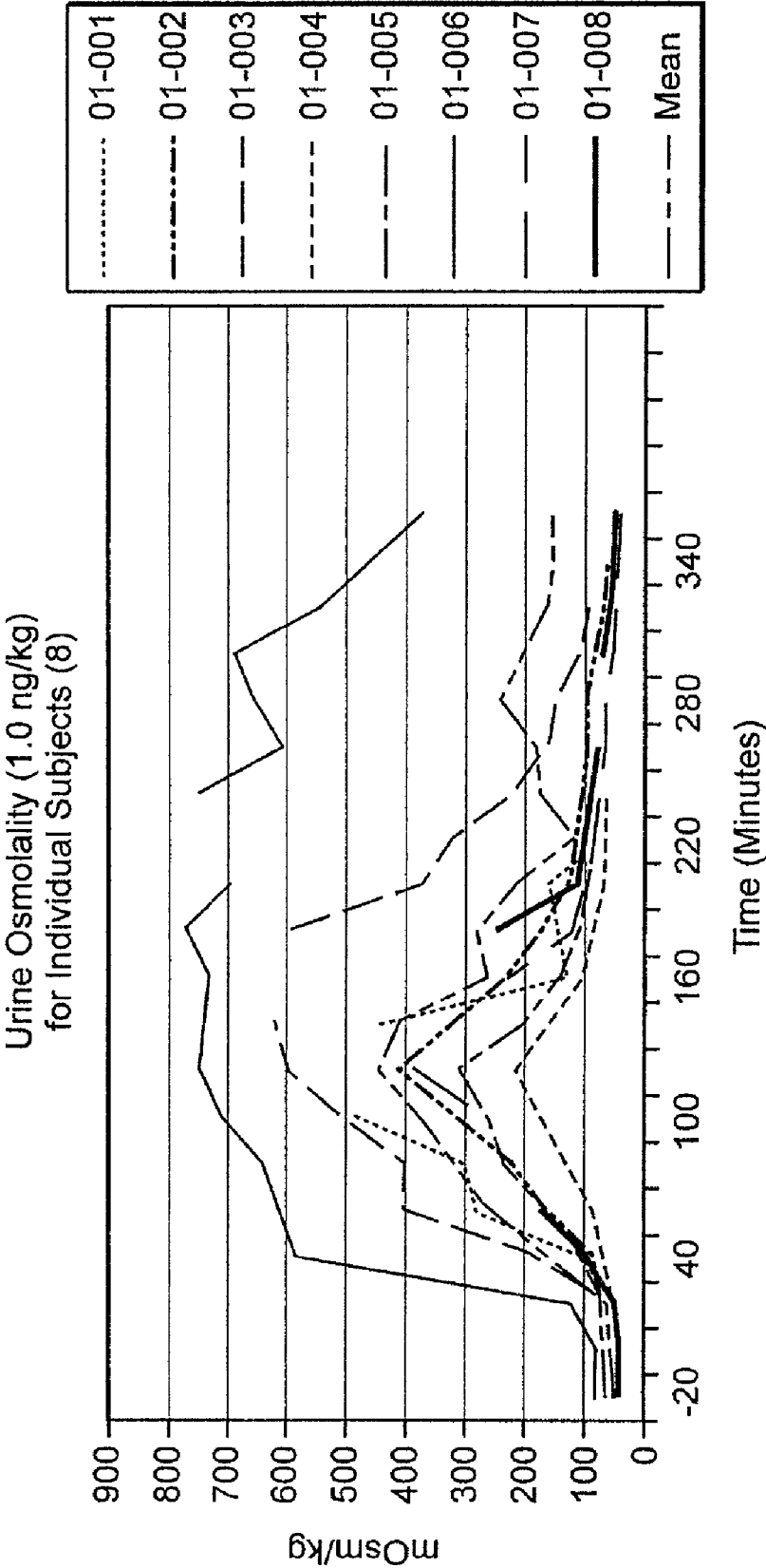


FIG. 2

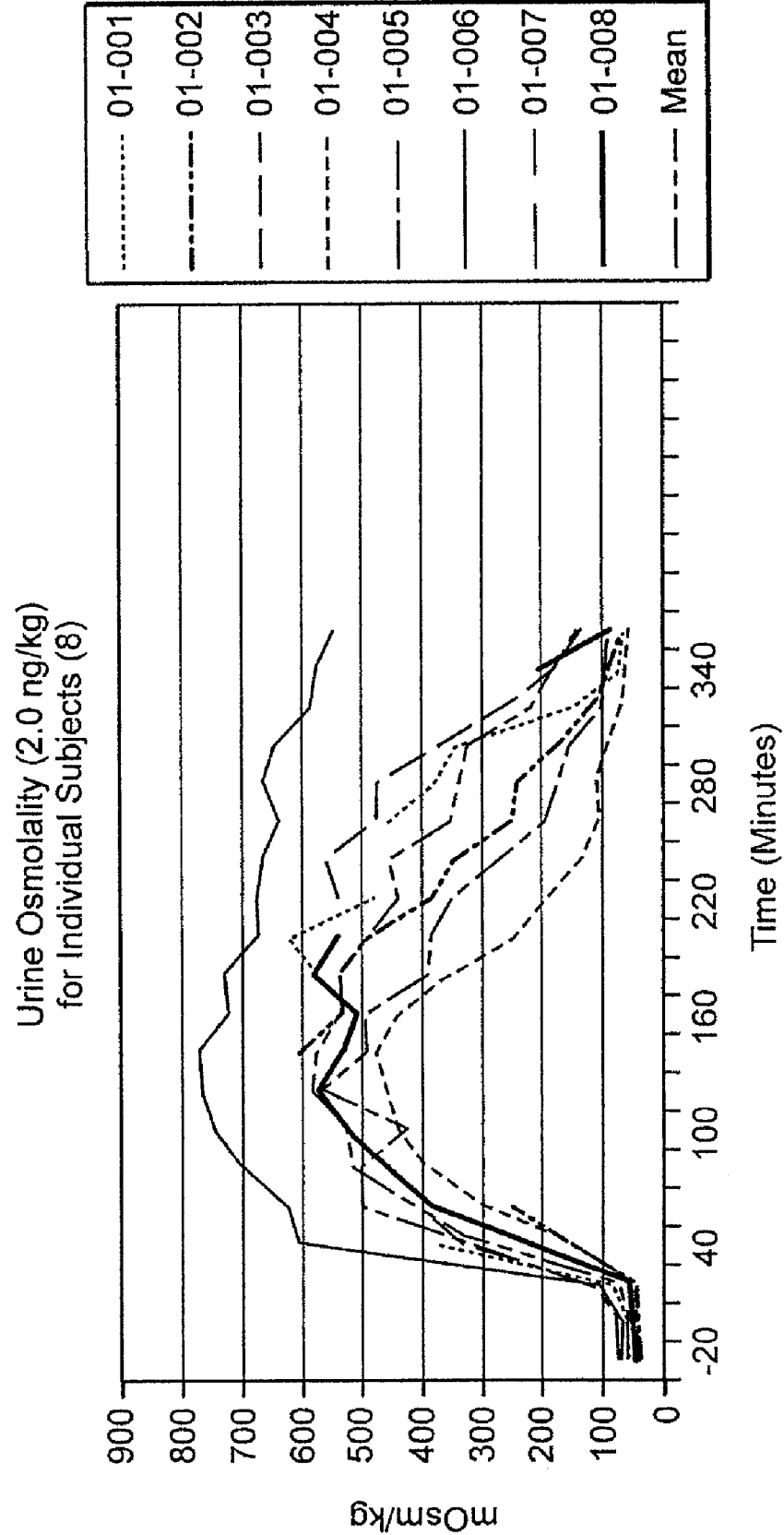


FIG. 3

Urine Output (0.5 ng/kg)
for Individual Subjects (8)

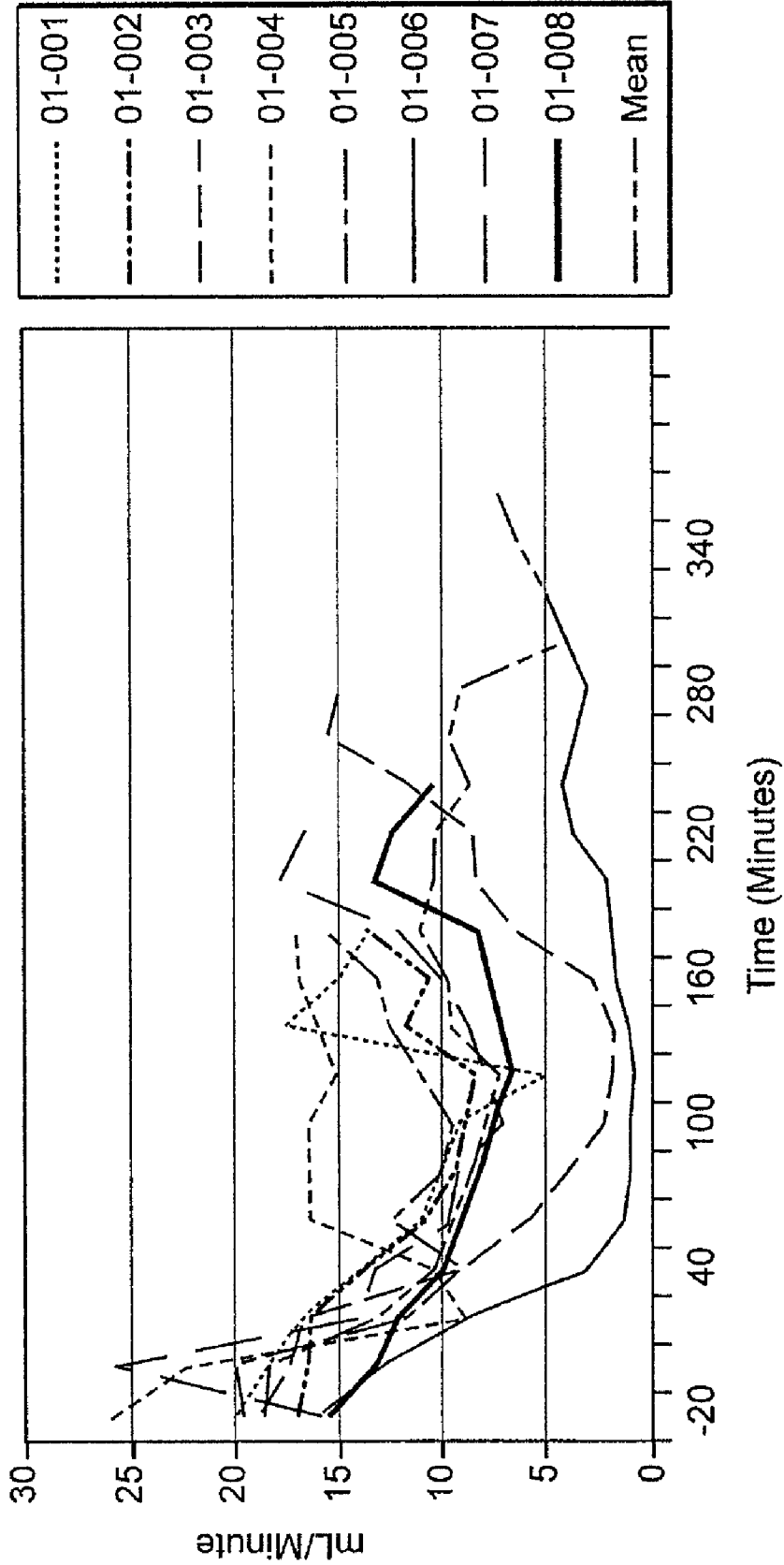


FIG. 4

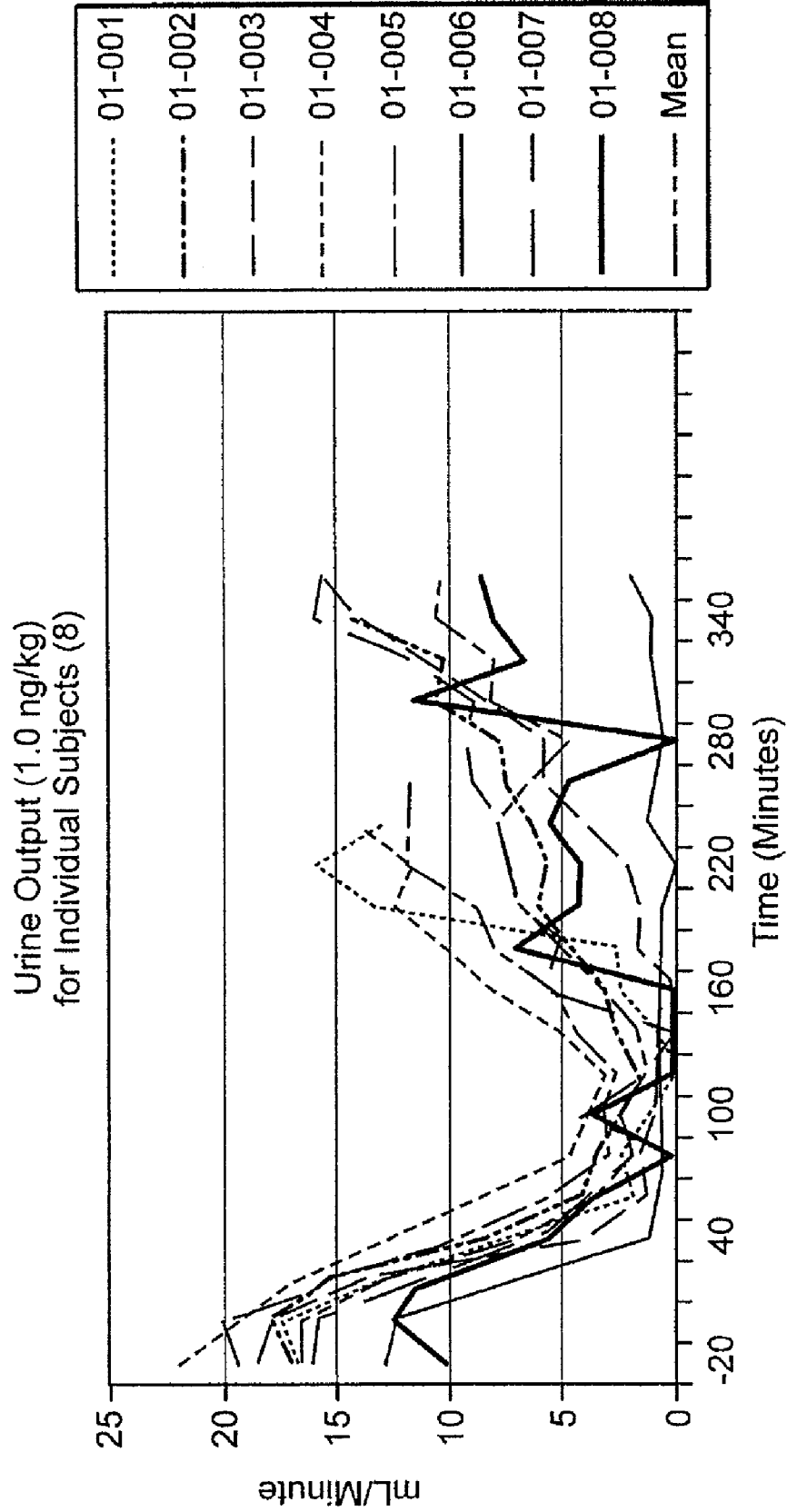


FIG. 5

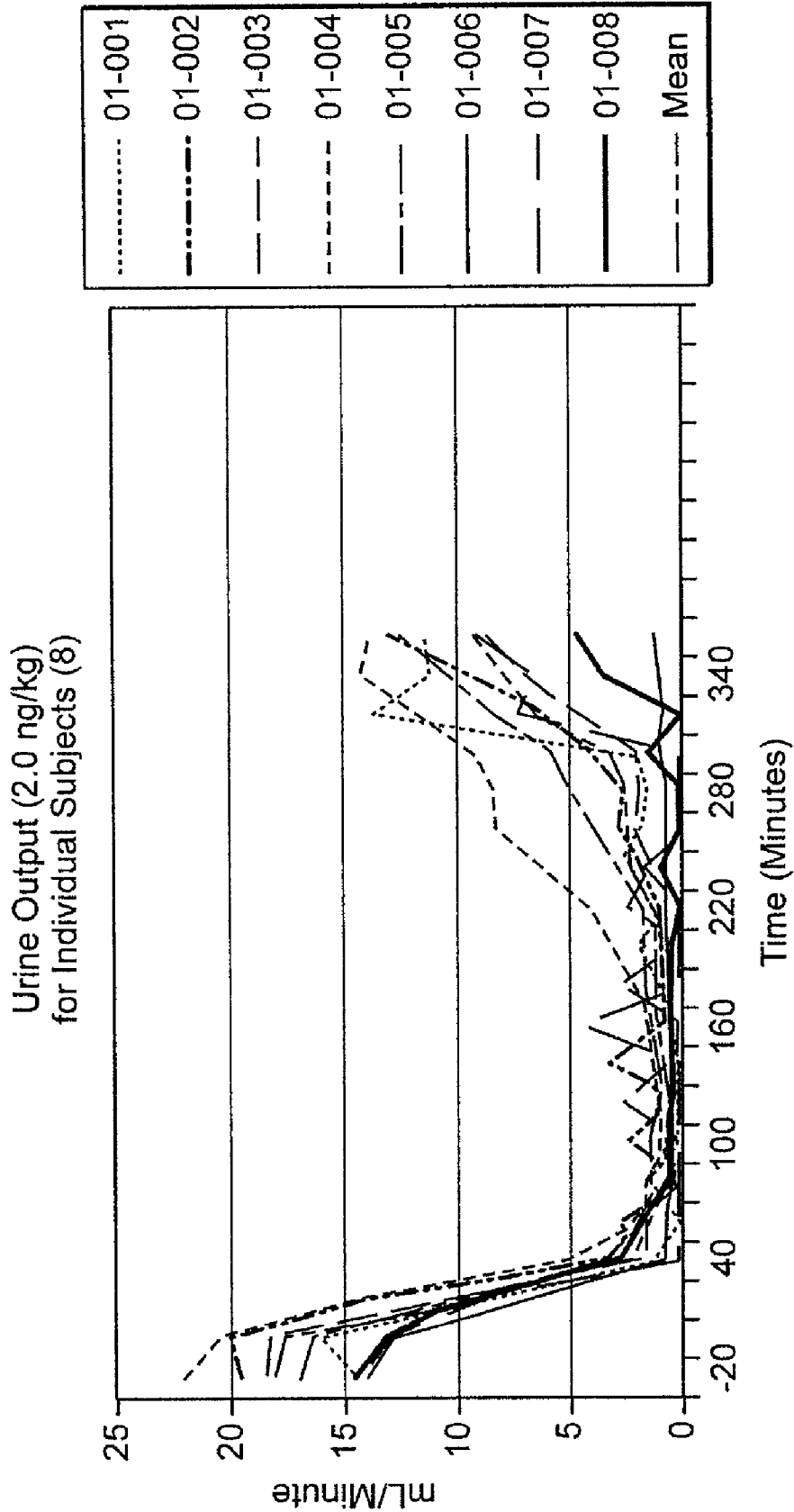


FIG. 6

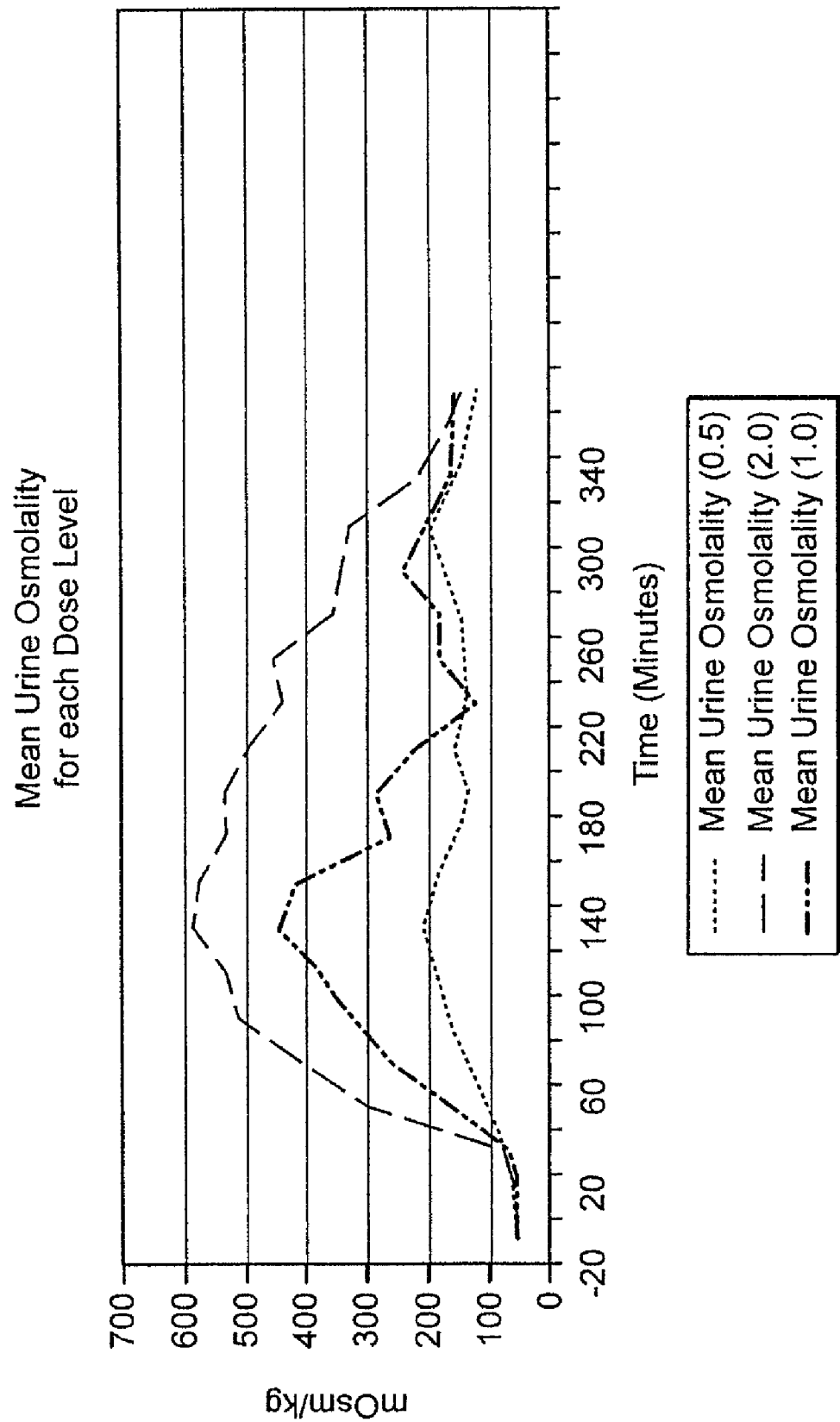


FIG. 7

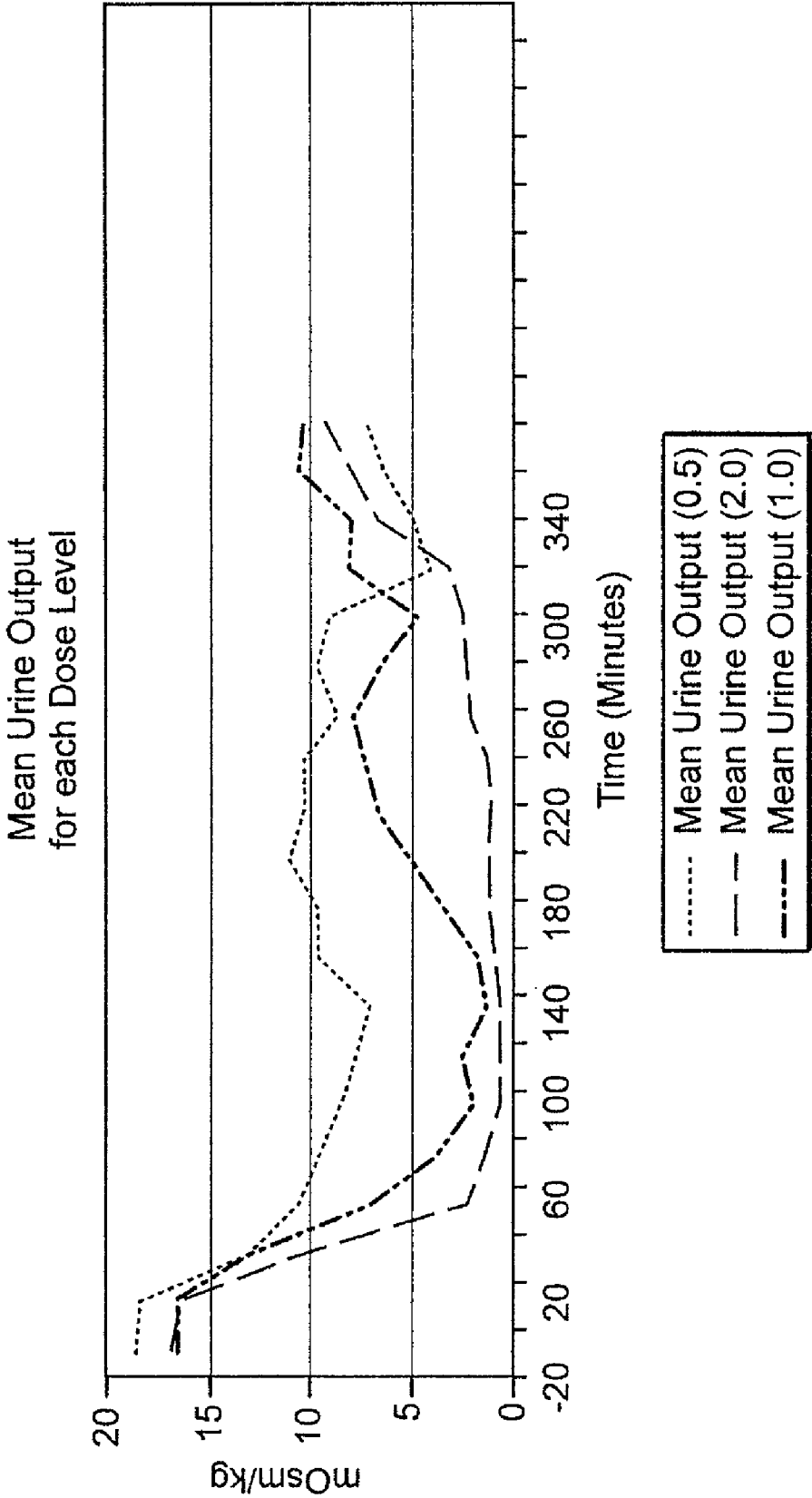


FIG. 8

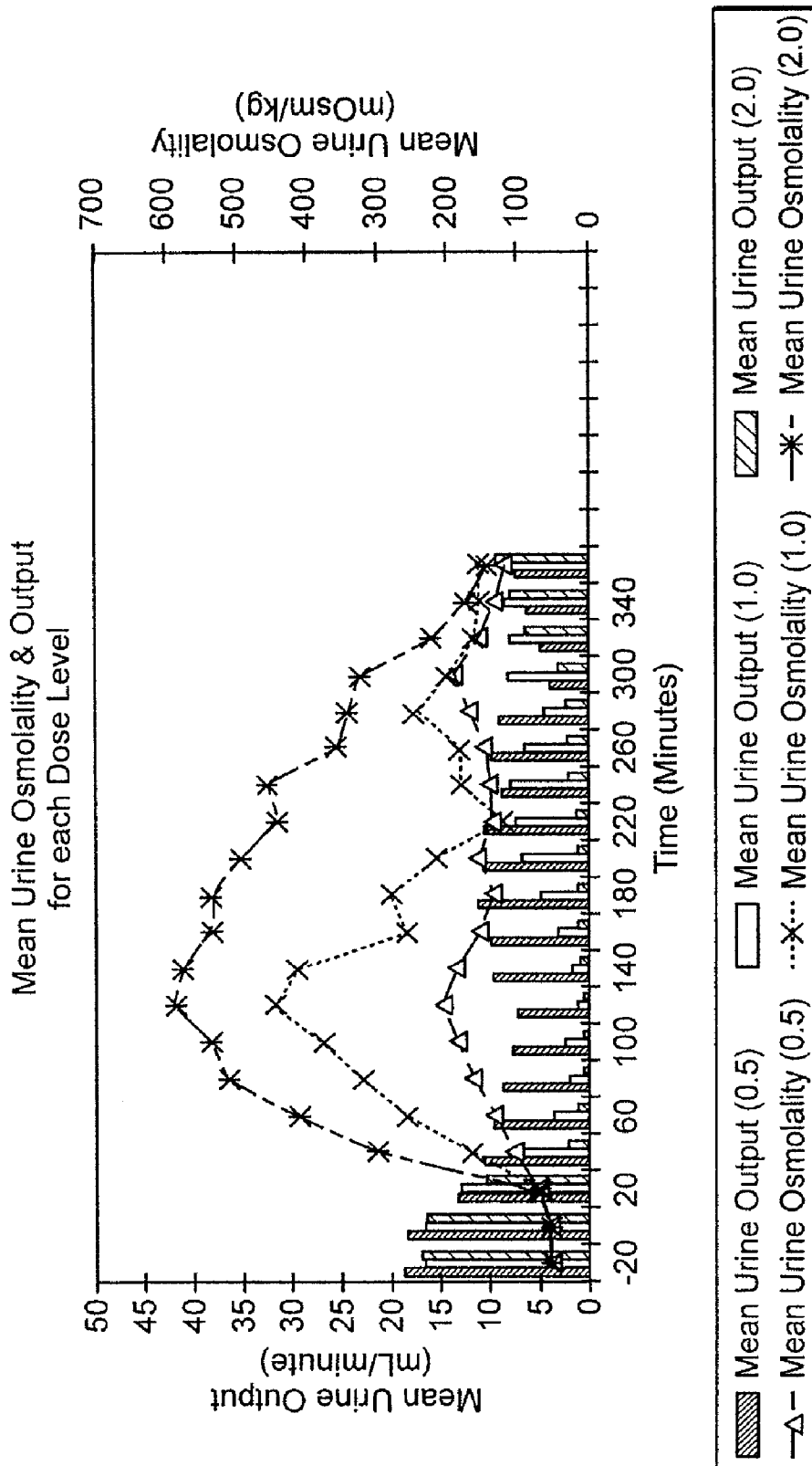


FIG. 9

1

PHARMACEUTICAL COMPOSITIONS INCLUDING LOW DOSAGES OF DESMOPRESSIN

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of co-pending application Ser. No. 10/706,100, filed Nov. 12, 2003; which is a Continuation-In-Part Application of PCT Application PCT/US03/14463 filed May 6, 2003. All of the aforementioned patent applications are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to pharmaceutical compositions including desmopressin, and more particularly to pharmaceutical compositions including low dosages of desmopressin for treatment of certain human diseases.

2. Brief Description of the Related Art

Desmopressin (1-desamino-8-D-arginine vasopressin, dDAVP) is an analogue of vasopressin. Desmopressin has decreased vasopressor activity and increased antidiuretic activity compared to vasopressin. This pharmacological profile enables desmopressin to be clinically used for antidiuresis without causing significant increases in blood pressure. Desmopressin is commercially available as the acetate salt both in tablet form and as a nasal spray, and is commonly prescribed for voiding postponement, incontinence, primary nocturnal enuresis (PNE) and nocturia, among other indications, including central diabetes insipidus.

Desmopressin has been administered intravenously, subcutaneously, intranasally and orally. The intravenous route of administration is clinically used almost exclusively to treat patients with mild hemophilia or Von Willebrand's Disease to raise blood levels of Factor VIII prior to surgery. Subcutaneous injection is used infrequently and primarily in patients with central diabetes insipidus, a deficiency of vasopressin resulting in the renal production of large volumes of extremely dilute urine which can cause severe dehydration. Intranasal administration of desmopressin via a nasal spray is approved for the maintenance treatment of patients with central diabetes insipidus and in children (ages 6 to 16 years) with primary nocturnal enuresis. An oral tablet dosage form of desmopressin is also approved for the treatment of central diabetes insipidus and primary nocturnal enuresis.

Currently, approved labeling for desmopressin recommends dosing in the following ranges depending on the clinical indication and the route of administration:

| Clinical Indication | Route of Administration (% Bioavailability) | Dose Range (daily) |
|----------------------------------|--|--|
| Hemophilia/Von Willebrand's | Intravenous (100) | 0.3 mcg/kg (21 mcg for 70 kg patients) |
| Central Diabetes Insipidus | Intravenous (100) | 2-4 mcg qd or 1-2 mcg bid |
| (CDI) | Subcutaneous (± 90) | 2-4 mcg qd or 1-2 mcg bid |
| | Intranasal (3-5) | 5-40 mcg qd or 5-20 mcg bid |
| | Oral (0.1) | 100-600 mcg bid |
| Primary Nocturnal Enuresis (PNE) | Intranasal (3-5) | 10-40 mcg qhs |
| | Oral (0.1) | 200-600 mcg qhs |

The maximum plasma/plasma/serum concentrations achieved with a typical intranasal dose of desmopressin for

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CDI or PNE of 20 micrograms (mcg or μg) would be approximately 20-30 pg/mL based on 3-5% bioavailability. For the desmopressin oral tablet with only 0.1-0.15% bioavailability, a standard dose of 200-400 mcg would also produce a peak plasma/plasma/serum level of 20-30 pg/mL.

While existing formulations of desmopressin have met the needs of patients, there is still a need for improvement. Tablets are often preferred by patients because of their ease of use, discretion and the lack of uncertainty of correct administration. However, tablets generally need to be taken with a glass of water or other drink, which is a problem as fluid intake needs to be restricted in connection with desmopressin treatment, and the message to the patient is much clearer when there is no water intake at all. In addition, while the above doses and plasma/plasma/serum concentrations are effective for treating CDI and PNE, standard dosages of desmopressin have been shown to cause undesirable side-effects including high incidences of hyponatremia. Lower dosages are preferable if the same desired effect could be produced. However, the current trend in this field is the evaluation of higher dosages of desmopressin for treatment purposes.

SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to a pharmaceutical composition, comprising 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

In another aspect, the present invention is directed to a pharmaceutical composition, comprising desmopressin and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is effective to establish a steady plasma/plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/plasma/serum to about 10.00 picogram desmopressin per mL plasma/plasma/serum.

In another aspect, the present invention is directed to an article of manufacture comprising packaging material and a pharmaceutical composition contained within the packaging material, wherein the pharmaceutical composition is therapeutically effective for treating or preventing hemophilia, Von Willebrand's Disease, incontinence, primary nocturnal enuresis (PNE), nocturia, or central diabetes insipidus, and wherein the packaging material comprises a label which indicates that the pharmaceutical composition can be used for treating or preventing hemophilia, Von Willebrand's Disease, incontinence, primary nocturnal enuresis (PNE), nocturia, or central diabetes insipidus, and wherein the pharmaceutical composition comprises 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

In another aspect, the present invention is directed to a method of treating or preventing a disease or condition which is treatable or preventable by desmopressin, the method comprising administering to a patient a daily dose of a therapeutically effective amount of a pharmaceutical composition comprising 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

In another aspect, the present invention is directed to methods of inducing antidiuretic effects in a patient, comprising the step of administering to a patient a daily dose of a therapeutically effective amount of a pharmaceutical composition comprising 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

These and other aspects will become apparent upon reading the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

The invention will be more fully understood from the following detailed description taken in conjunction with the accompanying figures in which:

FIG. 1 shows urine osmolality for each subject as a result of administration of 0.5 ng/kg of desmopressin;

FIG. 2 shows urine osmolality for each subject as a result of administration of 1.0 ng/kg of desmopressin;

FIG. 3 shows urine osmolality for each subject as a result of administration of 2.0 ng/kg of desmopressin;

FIG. 4 shows urine output for each subject as a result of administration of 0.5 ng/kg of desmopressin;

FIG. 5 shows urine output for each subject as a result of administration of 1.0 ng/kg of desmopressin;

FIG. 6 shows urine output for each subject as a result of administration of 2.0 ng/kg of desmopressin;

FIG. 7 shows mean urine osmolality resulting from administration of 0.5, 1.0, and 2.0 ng/kg desmopressin;

FIG. 8 shows urine output resulting from administration of 0.5, 1.0, and 2.0 ng/kg desmopressin; and

FIG. 9 shows mean urine osmolality and mean urine output resulting from administration of 0.5, 1.0, and 2.0 ng/kg desmopressin.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that desmopressin can be administered as a solid dosage form which is absorbed from the mouth and which provides improved bioavailability. It is surprising that desmopressin can be absorbed at all in this way, since the available evidence showed that desmopressin administered in the oral cavity (sub-lingually) was not significantly absorbed (Fjellestad-Paulsen A. et al., *Clin. Endocrinol.* 38 177-82 (1993)). It is even more unexpected that bioavailability can be improved compared to a conventional per oral tablet formulation (i.e. which is swallowed by the patient).

It has also been unexpectedly discovered that low doses and plasma/plasma/serum levels of desmopressin are pharmacologically active and can achieve desired therapeutic efficacy. The present inventor has found that doses and plasma/plasma/serum concentrations of desmopressin which are from 5 to 40% of the current recommended doses and resulting plasma/plasma/serum levels are therapeutically effective, and in some cases safer for treatment of CDI, PNE, and additional clinical indications requiring pharmacological concentration of the urine. It has been discovered that the actual dose response curve of desmopressin is shifted to the left relative to current theory and practice and that at each plasma/plasma/serum concentration point over the dose range predicted an incremental pharmacological effect in terms of urine concentration is observed.

According to a first aspect of the invention, there is provided a pharmaceutical dosage form of desmopressin adapted for sublingual absorption.

The desmopressin may be in the form of the free base or a pharmaceutically or, where appropriate veterinarily, acceptable salt, or in any other pharmaceutically or veterinarily acceptable form. The acetate salt is particularly preferred.

The formulation will typically be solid. It may disperse rapidly in the mouth. Such formulations are termed 'orodispersible'. The formulation will typically comprise a suitable carrier for this purpose, which will be pharmaceutically acceptable (or veterinarily acceptable in the case of administration to non-human animals).

The daily dosage of desmopressin, measured as the free base, will generally be from 0.5 or 1 µg to 1 mg per dosage form. In one preferred dosage range, the dosage will typically range from 2 µg to 800 µg per dosage form and preferably from 10 µg to 600 µg. Comparatively lower doses (e.g., lower dosages relative to the dosages above or provided in the art) are also specifically contemplated, for example from 0.5 ng to 20,000 ng, preferably 0.05 mcg (50 ng) to 10 mcg (10,000 ng), and more preferably 0.1 mcg (100 ng) to 2000 ng. When one dosage form per day is administered, as is usual for PNE and nocturia, this will typically be the dose per dosage form. When the daily dose is administered in two or more dosages, as will typically be the case for central diabetes insipidus, the amount of the active compound per dosage form will be reduced accordingly. The effective daily dosage will depend on the condition of the individual patient, and is thus within the ordinary skill of the art to determine for any particular patient. Other active ingredients, whether or not peptides, may also be present.

Pharmaceutical dosage forms of the present invention are adapted to supply the active ingredient to the oral cavity. The active may be absorbed across the sublingual mucosa for systemic distribution.

A variety of formulations are known which are suitable for delivering other active ingredients for absorption from the oral cavity. Such formulations may be useful in the present invention. Among them are intrabuccally disintegrating solid formulations or preparations which comprise the active ingredient, a sugar comprising lactose and/or mannitol and 0.12 to 1.2 w/w %, based on the solid components, of agar and which has a density of 400 mg/ml to 1,000 mg/ml and have a sufficient strength for handling, which in practice may mean sufficient strength to withstand removal from a blister packaging without disintegrating. Such formulations, and how to make them, are disclosed in U.S. Pat. No. 5,466,464, to which reference is made for further details.

In this embodiment of the invention, the sugar may be used in the formulation in an amount of at least 50 w/w %, preferably 80 w/w % or more, more preferably 90 w/w % or more, based on the total solid components, although it may vary depending on the quality and the quantity of the active ingredient to be used.

Though types of agar are not particularly limited, those listed in the Japanese Pharmacopoeia may be used preferably. Examples of the listed agar include agar powders PS-7 and PS-8 (manufactured by Ina Shokuhin).

Agar may be used in an amount from 0.12 to 1.2 w/w %, preferably from 0.2 to 0.4 w/w %, based on the solid components.

In order to produce a formulation in accordance with this embodiment of the present invention, a sugar comprising lactose and/or mannitol is suspended in an aqueous agar solution, filled with a mould, solidified into a jelly-like form and then dried. The aqueous agar solution may have a concentration of from 0.3 to 2.0%, preferably from 0.3 to 0.8%. The aqueous agar solution may be used in such an amount that the blending ratio of agar based on the solid components becomes 0.12 to 1.2 w/w %, but preferably 40 to 60 w/w % of agar solution based on the solid components.

Other formulations known for delivering active ingredients for absorption from the oral cavity are the dosage forms disclosed in U.S. Pat. No. 6,024,981 and U.S. Pat. No. 6,221,392. They are hard, compressed, rapidly dissolvable dosage forms adapted for direct oral dosing comprising: an active ingredient and a matrix including a non-direct compression filter and a lubricant, said dosage form being adapted to rapidly dissolve in the mouth of a patient and thereby liberate

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said active ingredient, and having a friability of about 2% or less when tested according to the U.S.P., said dosage form optionally having a hardness of at least about 15 Newtons (N), preferably from 15-50 N. U.S. Pat. No. 6,024,981 and U.S. Pat. No. 6,221,392 disclose further details and characteristics of these dosage forms and how to make them.

Preferably, dosage forms in accordance with this embodiment of the invention dissolve in about 90 seconds or less (preferably 60 seconds or less and most preferably 45 seconds or less) in the patient's mouth. It is also often desirable that the dosage form include at least one particle. The particle would be the active ingredient and a protective material. These particles can include rapid release particles and or sustained release particles.

In a particularly preferred formulation in accordance with this embodiment of the present invention there is provided a hard, compressed, rapidly dissolving tablet adapted for direct oral dosing. The tablet includes particles made of an active ingredient and a protective material. These particles are provided in an amount of between about 0.01 and about 75% by weight based on the weight of the tablet. The tablet also includes a matrix made from a non-direct compression filler, a wicking agent, and a hydrophobic lubricant. The tablet matrix comprises at least about 60% rapidly water soluble ingredients based on the total weight of the matrix material. The tablet has a hardness of between about 15 and about 50 Newtons, a friability of less than 2% when measured by U.S.P. and is adapted to dissolve spontaneously in the mouth of a patient in less than about 60 seconds and thereby liberate said particles and be capable of being stored in bulk.

A very fine grained or powdered sugar known as a non-direct compression sugar may be used as a filler in the matrix of this embodiment the present invention. This material, in part because of its chemical composition and in part because of its fine particle size, will dissolve readily in the mouth in a matter of seconds once it is wetted by saliva. Not only does this mean that it can contribute to the speed at which the dosage form will dissolve, it also means that while the patient is holding the dissolving dosage form in his or her mouth, the filler will not contribute a "gritty" or "sandy" texture thus adversely affecting the organoleptic sensation of taking the dosage form. In contrast, direct compression versions of the same sugar are usually granulated and treated to make them larger and better for compaction. While these sugars are water soluble, they may not be solubilised quickly enough. As a result, they can contribute to the gritty or sandy texture of the dosage form as it dissolves. Dissolution time in the mouth can be measured by observing the dissolution time of the tablet in water at about 37° C. The tablet is immersed in the water without forcible agitation or with minimal agitation. The dissolution time is the time from immersion to substantially complete dissolution of the rapidly water soluble ingredients of the tablet as determined by visual observation.

Particularly preferred fillers, in accordance with the present invention are non-direct compression sugars and sugar alcohols which meet the specifications discussed above. Such sugars and sugar alcohols include, without limitation, dextrose, mannitol, sorbitol, lactose and sucrose. Of course, dextrose, for example, can exist as either a direct compression sugar, i.e., a sugar which has been modified to increase its compressibility, or a non-direct compression sugar.

Generally, the balance of the formulation can be matrix. Thus the percentage of filler can approach 100%. However, generally, the amount of non-direct compression filler useful in accordance with the present invention ranges from about

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25 to about 95%, preferably between about 50 and about 95% and more preferably from about 60 to about 95%.

The amount of lubricant used can generally range from between about 1 to about 2.5% by weight, and more preferably between about 1.5 to about 2% by weight. Hydrophobic lubricants useful in accordance with the present invention include alkaline stearates, stearic acid mineral and vegetable oils, glyceryl behenate and sodium stearyl fumarate. Hydrophilic lubricants can also be used.

Protective materials useful in accordance with this embodiment of the present invention may include any of the polymers conventionally utilized in the formation of microparticles, matrix-type microparticles and microcapsules. Among these are cellulosic materials such as naturally occurring cellulose and synthetic cellulose derivatives; acrylic polymers and vinyl polymers. Other simple polymers include proteinaceous materials such as gelatin, polypeptides and natural and synthetic shellacs and waxes. Protective polymers may also include ethylcellulose, methylcellulose, carboxymethyl cellulose and acrylic resin material sold under the registered trade mark EUDRAGIT by Rhone Pharma GmbH of Weierstadt, Germany.

In addition to the ingredients previously discussed, the matrix may also include wicking agents, non-effervescent disintegrants and effervescent disintegrants. Wicking agents are compositions which are capable of drawing water up into the dosage form. They help transport moisture into the interior of the dosage form. In that way the dosage form can dissolve from the inside, as well as from the outside.

Any chemical which can function to transport moisture as discussed above can be considered a wicking agent. Wicking agents include a number of traditional non-effervescent disintegration agents. These include, for example, microcrystalline cellulose (AVICEL PH 200, AVICEL PH 101), Ac-Di-Sol (Croscarmellose Sodium) and PVP-XL (a crosslinked polyvinylpyrrolidone); starches and modified starches, polymers, and gum such as Arabic and xanthan. Hydroxyalkyl cellulose such as hydroxymethylcellulose, hydroxypropylcellulose and hydroxypropylmethylcellulose, as well as compounds such as carbopol may be used as well.

The conventional range of non-effervescent disintegrant agents used in conventional tablets can be as high as 20%. However, generally, the amount of disintegration agent used ranged from between about 2 and about 5%, according to the Handbook of Pharmaceutical Excipients.

In accordance with this embodiment of the present invention, the amount of wicking agents used may range from between 2 to about 12% and preferably from between 2 to about 5%.

It is also possible, of course, to include non-effervescent disintegrants which may not act to wick moisture, if desirable. In either event, it is preferable to use either rapidly water soluble, non-effervescent disintegrants or wicking agents and/or to minimize the use of generally non-water soluble wicking agents or non-effervescent disintegrants. Non-rapidly dissolvable, non-rapidly water soluble elements if used in sufficient quantity, can adversely affect the organoleptic properties of the tablets as they dissolve within the mouth and therefore should be minimized. Of course, wicking agents or non-effervescent disintegrants which are rapidly water soluble as discussed herein can be used in greater quantity and they will not add to the grittiness of the formulation during dissolution. Preferred wicking agents in accordance with the present invention include crosslinked PVP, although, the amounts of these must be controlled as they are not rapidly water soluble.

In addition, it may be desirable to use an effervescent couple, in combination with the other recited ingredients to improve the disintegration profile, the organoleptic properties of the material and the like. Preferably, the effervescent couple is provided in an amount of between about 0.5 and about 50%, and more preferably, between about 3 and about 15% by weight, based on the weight of the finished tablet. It is particularly preferred that sufficient effervescent material be provided such that the evolved gas is less than about 30 cm³, upon exposure to an aqueous environment.

The term "effervescent couple" includes compounds which evolve gas. The preferred effervescent couple evolve gas by means of a chemical reaction which takes place upon exposure of the effervescent disintegration couple to water and/or to saliva in the mouth. This reaction is most often the result of the reaction of a soluble acid source and an alkali monohydrogencarbonate or other carbonate source. The reaction of these two general compounds produces carbon dioxide gas upon contact with water or saliva. Such water-activated materials must be kept in a generally anhydrous state and with little or no absorbed moisture or in a stable hydrated form, since exposure to water will prematurely disintegrate the tablet. The acid sources may be any which are safe for human consumption and may generally include food acids, acid and hydrite antacids such as, for example: citric, tartaric, malic, fumaric, adipic, and succinics. Carbonate sources include dry solid carbonate and bicarbonate salt such as, preferably, sodium bicarbonate, sodium carbonate, potassium bicarbonate and potassium carbonate, magnesium carbonate and the like. Reactants which evolve oxygen or other gasses and which are safe for human consumption are also included.

In the case of the orally dissolvable tablets in accordance with the present invention, it is preferred that both the amount and the type of disintegration agent, either effervescent or non-effervescent, and the combination thereof be provided sufficient in a controlled amount such that the tablet provides a pleasant organoleptic sensation in the mouth of the patient. In some instances, the patient should be able to perceive a distinct sensation of fizzing or bubbling as the tablet disintegrates in the mouth. In general, the total amount of wicking agents, non-effervescent disintegrants and effervescent disintegrants should range from 0-50%. However, it should be emphasized that the formulations of the present invention will dissolve rapidly and therefore, the need for disintegrating agents is minimal. As illustrated in the examples, appropriate hardness, friability and dissolution times can be obtained even without effervescent disintegrants or high quantities of wicking agents.

The use of a non-direct compression filler eliminates the need for many conventional processing steps such as granulation and/or the need to purchase more expensive pre-granulated, compressible fillers. At the same time, the resulting dosage form is a balance of performance and stability. It is robust enough to be conventionally produced using direct compression. It is robust enough to be stored or packaged in bulk. Yet, it rapidly dissolves in the mouth while minimizing the unpleasant feel of conventional disintegrating tablets to the extent possible.

Formulations in accordance with the embodiment of the invention may be made by a method including the steps of:

(a) forming a mixture including an active ingredient and a matrix including a non-direct compression filler and a lubricant;

(b) compressing the mixture to form a plurality of hard, compressed, rapidly disintegrable dosage forms including the active ingredient distributed in the orally dissolvable matrix; and optionally

(c) storing the dosage forms in bulk prior to packaging. In a preferred embodiment, the dosage forms are then packaged in a lumen of a package such that there is at least one per package. In a preferred particularly preferred embodiment, the dosage forms are then packaged in a lumen of a package such that there more than one per package. Direct compression is the preferred method of forming the dosage forms.

Other formulations known for delivering active ingredients for absorption from the oral cavity are the dosage forms disclosed in U.S. Pat. No. 6,200,604, which comprise an orally administrable medicament in combination with an effervescent agent used as penetration enhancer to influence the permeability of the medicament across the buccal, sublingual, and gingival mucosa. In the content of the present invention, the medicament is desmopressin, which is administered in most embodiments across the sublingual mucosa. In the formulations of this embodiment of the invention, effervescent agents can be used alone or in combination with other penetration enhancers, which leads to an increase in the rate and extent of oral absorption of an active drug.

Formulations or dosage forms in accordance with this embodiment of the invention should include an amount of an effervescent agent effective to aid in penetration of the drug across the oral mucosa. Preferably, the effervescent is provided in an amount of between about 5% and about 95% by weight, based on the weight on the finished tablet, and more preferably in an amount of between about 30% and about 80% by weight. It is particularly preferred that sufficient effervescent material be provided such that the evolved gas is more than about 5 cm³ but less than about 30 cm³, upon exposure of the tablet to an aqueous environment.

The term "effervescent agent" includes compounds which evolve gas. The preferred effervescent agents evolve gas by means of a chemical reaction which takes place upon exposure of the effervescent agent (an effervescent couple) to water and/or to saliva in the mouth. This reaction is most often the result of the reaction of a soluble acid source and a source of carbon dioxide such as an alkaline carbonate or bicarbonate. The reaction of these two general compounds produces carbon dioxide gas upon contact with water or saliva. Such water-activated materials must be kept in a generally anhydrous state and with little or no absorbed moisture or in a stable hydrated form, since exposure to water will prematurely disintegrate the tablet. The acid sources may be any which are safe for human consumption and may generally include food acids, acid and hydrite antacids such as, for example: citric, tartaric, malic, fumaric, adipic, and succinics. Carbonate sources include dry solid carbonate and bicarbonate salt such as, preferably, sodium bicarbonate, sodium carbonate, potassium bicarbonate and potassium carbonate, magnesium carbonate and the like. Reactants which evolve oxygen or other gasses and which are safe for human consumption are also included.

The effervescent agent(s) useful in this embodiment of the present invention is not always based upon a reaction which forms carbon dioxide. Reactants which evolve oxygen or other gasses which are safe for human consumption are also considered within the scope. Where the effervescent agent includes two mutually reactive components, such as an acid source and a carbonate source, it is preferred that both components react completely. Therefore, an equivalent ratio of components which provides for equal equivalents is preferred. For example, if the acid used is diprotic, then either

twice the amount of a mono-reactive carbonate base, or an equal amount of a di-reactive base should be used for complete neutralization to be realized. However, in other embodiments of the present invention, the amount of either acid or carbonate source may exceed the amount of the other component. This may be useful to enhance taste and/or performance of a tablet containing an overage of either component. In this case, it is acceptable that the additional amount of either component may remain unreacted.

Such dosage forms may also include the amounts additional to that required for effervescence a pH adjusting substance. For drugs that are weakly acidic or weakly basic, the pH of the aqueous environment can influence the relative concentrations of the ionized and unionized forms of the drug present in solution according to the Henderson-Hasselbach equation. The pH solutions in which an effervescent couple has dissolved is slightly acidic due to the evolution of carbon dioxide. The pH of the local environment, e.g. saliva in immediate contact with the tablet and any drug that may have dissolved from it, may be adjusted by incorporating in the tablet a pH adjusting substances which permit the relative portions of the ionized and unionized forms of the drug to be controlled. In this way, the present dosage forms can be optimized for each specific drug. If the unionized drug is known or suspected to be absorbed through the cell membrane (transcellular absorption) it would be preferable to alter the pH of the local environment (within the limits tolerable to the subject) to a level that favours the unionized form of the drug. Conversely, if the ionized form is more readily dissolved the local environment should favour ionization.

The aqueous solubility of the drug should preferably not be compromised by the effervescent and pH adjusting substance, such that the dosage forms permit a sufficient concentration of the drug to be present in the unionized form. The percentage of the pH adjusting substance and/or effervescent should therefore be adjusted depending on the drug.

Suitable pH adjusting substance for use in the present invention include any weak acid or weak base in amounts additional to that required for the effervescence or, preferably, any buffer system that is not harmful to the oral mucosa. Suitable pH adjusting substance for use in the present invention include, but are not limited to, any of the acids or bases previously mentioned as effervescent compounds, disodium hydrogen phosphate, sodium dihydrogen phosphate and the equivalent potassium salt.

The dosage form of this embodiment of the invention preferably includes one or more other ingredients to enhance the absorption of the pharmaceutical ingredient across the oral mucosa and to improve the disintegration profile and the organoleptic properties of the dosage form. For example, the area of contact between the dosage form and the oral mucosa, and the residence time of the dosage form in the oral cavity can be improved by including a bioadhesive polymer in this drug delivery system. See, for example, Mechanistic Studies on Effervescent-Induced Permeability Enhancement by Jonathan Eichman (1997), which is incorporated by reference herein. Effervescence, due to its mucus stripping properties, would also enhance the residence time of the bioadhesive, thereby increasing the residence time for the drug absorption. Non-limiting examples of bioadhesives used in the present invention include, for example, Carbopol 934 P, Na CMC, Methocel, Polycarbophil (Noveon AA-1), HPMC, Na alginate, Na Hyaluronate and other natural or synthetic bioadhesives.

In addition to the effervescence-producing agents, a dosage form according to this embodiment of the present invention may also include suitable non-effervescent disintegra-

tion agents. Non-limiting examples of non-effervescent disintegration agents include: microcrystalline, cellulose, croscarmellose sodium, crospovidone, starches, corn starch, potato starch and modified starches thereof, sweeteners, clays, such as bentonite, alginates, gums such as agar, guar, locust bean, karaya, pectin and tragacanth. Disintegrants may comprise up to about 20 weight percent and preferably between about 2 and about 10% of the total weight of the composition.

In addition to the particles in accordance with this embodiment of the present invention, the dosage forms may also include glidants, lubricants, binders, sweeteners, flavouring and colouring components. Any conventional sweetener or flavouring component may be used. Combinations of sweeteners, flavouring components, or sweeteners and flavouring components may likewise be used.

Examples of binders which can be used include acacia, tragacanth, gelatin, starch, cellulose materials such as methyl cellulose and sodium carboxy methyl cellulose, alginic acids and salts thereof, magnesium, aluminium silicate, polyethylene glycol, guar gum, polysaccharide acids, bentonites, sugars, invert sugars and the like. Binders may be used in an amount of up to 60 weight percent and preferably about 10 to about 40 weight percent of the total composition.

Colouring agents may include titanium dioxide, and dyes suitable for food such as those known as F.D. & C. dyes and natural coloring agents such as grape skin extract, beet red powder, beta-carotene, annatto, carmine, turmeric, paprika, etc. The amount of colouring used may range from about 0.1 percent to about 3.5 weight percent of the total composition.

Flavours incorporated in the composition may be chosen from synthetic flavours oils and flavouring aromatics and/or natural oils, extracts from plants, leaves, flowers, fruits and so forth and combinations thereof. These may include cinnamon oil, oil of wintergreen, peppermint oils, clove oil, bay oil anise oil, eucalyptus, thyme oil, cedar leave oil, oil of nutmeg, oil of sage, oil of bitter almonds and cassia oil. Also useful as flavours are vanilla, citrus oil, including lemon, orange, grape, lime and grapefruit, and fruit essences, including apple, pear, peach, strawberry, raspberry, cherry, plum, pineapple, apricot and so forth. Flavours which have been found to be particularly useful include commercially available orange, grape, cherry and bubble gum flavours and mixtures thereof. The amount of flavouring may depend on a number of factors, including the organoleptic effect desired. Flavours may be present in an amount ranging from about 0.05 to about 3 percent by weight based upon the weight of the composition. Particularly preferred flavours are the grape and cherry flavours and the citrus flavours such as orange.

One aspect of the invention provides a solid, oral tablet dosage form suitable for sublingual administration. Excipient fillers can be used to facilitate tableting. The filler desirably will also assist in the rapid dissolution of the dosage form in the mouth. Non-limiting examples of suitable fillers include: mannitol, dextrose, lactose, sucrose, and calcium carbonate.

As described in U.S. Pat. No. 6,200,604, tablets can either be manufactured by direct compression, wet granulation or any other tablet manufacturing technique. The dosage form may be administered to a human or other mammalian subject by placing the dosage form in the subject's mouth and holding it in the mouth, beneath the tongue (for sublingual administration). The dosage form spontaneously begins to disintegrate due to the moisture in the mouth. The disintegration, particularly the effervescence, stimulates additional salivation which further enhances disintegration.

Although the above described formulations are within the scope of the present invention, the most preferred orodispers-

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ible solid pharmaceutical dosage forms according to the invention comprise a pharmaceutically active peptide and an open matrix network carrying desmopressin, the open matrix network being comprised of a water-soluble or water-dispersible carrier material that is inert towards desmopressin.

Pharmaceutical dosage forms comprising open matrix networks are known from GB-A-1548022, to which reference is made for further details. Pharmaceutical dosage forms of the invention can be rapidly disintegrated by water. By "rapidly disintegrated" is meant that the shaped articles are disintegrated in water within 10 seconds. Preferably the shaped article disintegrates (dissolves or disperses) within 5 seconds or less. The disintegration time is measured by a procedure analogous to the Disintegration Test for Tablets, B.P. 1973. The procedure is described in GB-A-1548022 and outlined below.

Apparatus

A glass or suitable plastic tube 80 to 100 mm long, with an internal diameter of about 28 mm and an external diameter of 30 to 31 mm, and fitted at the lower end, so as to form a basket, with a disc of rustproof wire gauze complying with the requirements for a No. 1.70 sieve.

A glass cylinder with a flat base and an internal diameter of about 45 mm containing water not less than 15 cm deep at a temperature between 36° and 38° C.

The basket is suspended centrally in the cylinder in such a way that it can be raised and lowered repeatedly in a uniform manner so that at the highest position the gauze just breaks the surface of the water and at the lowest position the upper rim of the basket just remains clear of the water.

Method

Place one shaped article in the basket and raise and lower it in such a manner that the complete up and down movement is repeated at a rate equivalent to thirty times a minute. The shaped articles are disintegrated when no particle remains above the gauze which would not readily pass through it. No such particle should remain after 10 seconds.

By the term "open matrix network" there is meant a network of water-soluble or water-dispersible carrier material having interstices dispersed throughout. The open matrix network of carrier material is of generally low density. For example the density may be within the range 10 to 200 mg/cc e.g. 10 to 100 mg/cc, preferably 30 to 60 mg/cc. The density of the shaped article may be affected by the amount of active ingredient, or any other ingredients, incorporated into the article and may be outside the above mentioned preferred limits for the density of the matrix network. The open matrix network which is similar in structure to a solid foam enables a liquid to enter the product through the interstices and permeate through the interior. Permeation by aqueous media exposes the carrier material of both the interior and exterior of the product to the action of the aqueous media whereby the network of carrier material is rapidly disintegrated. The open matrix structure is of a porous nature and enhances disintegration of the product as compared with ordinary solid shaped pharmaceutical dosage forms such as tablets, pills, capsules, suppositories and pessaries. Rapid disintegration results in rapid release of the active ingredient carried by the matrix.

The carrier material used in the product of the invention may be any water-soluble or water-dispersible material that is pharmacologically acceptable or inert to the chemical and which is capable of forming a rapidly disintegratable open matrix network. It is preferred to use water-soluble material as the carrier since this results in the most rapid disintegration of the matrix when the product is placed in an aqueous medium. A particularly advantageous carrier may be formed

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from polypeptides such as gelatin, particularly gelatin which is particularly hydrolysed, e.g. by heating in water. For example, the gelatin may be partially hydrolysed by heating a solution of the gelatin in water, e.g. in an autoclave at about 120° C. for up to 2 hours, e.g. from about 5 minutes to about 1 hour, preferable from about 30 minutes to about 1 hour. The hydrolysed gelatin is preferably used at concentrations of about 1 to 6% weight/vol., most preferably at 2 to 4% e.g. about 3%.

Although mammalian derived gelatin may be used, it has an unpleasant taste and thus necessitates the use of sweeteners and flavours to mask the taste of the gelatin in addition to any sweeteners and flavours which may be required to mask the taste of the active ingredient. Moreover, the heating step necessary with the use of mammalian gelatin increases processing times and incurs heating costs thereby increasing the overall costs of the process. Therefore, the use of fish gelatin, especially non-gelling fish gelatin, is preferred, especially for desmopressin. Reference is made to WO-A-0061117 for further details.

Other carrier materials may be used in place of partially hydrolysed gelatin or fish gelatin, for example polysaccharides such as hydrolysed dextran, dextrin and alginates (e.g. sodium alginate) or mixtures of above mentioned carriers with each other or with other carrier materials such as polyvinyl alcohol, polyvinylpyrrolidone or acacia. Modified starch may also be used in place of gelatin, as described in WO-A-0044351, to which reference is made for further details. Additional carriers include water, lactose, starch, magnesium stearate, talc, plant oils, gums, alcohol, Vaseline (petroleum jelly), or the like.

Pharmaceutical dosage forms of the invention may be in the form of shaped articles. They may incorporate ingredients in addition to the active ingredient(s). For example the pharmaceutical dosage form of the present invention may incorporate pharmaceutically acceptable adjuvants. Such adjuvants include, for example, colouring agents, flavouring agents, preservatives (e.g. bacteriostatic agents), and the like. U.S. Pat. No. 5,188,825 teaches that water soluble active agents should be bonded to an ion exchange resin to form a substantially water insoluble active agent/resin complex; although that teaching may be practiced here (for which reference to U.S. Pat. No. 5,188,825 is made for further details), it has been found in the development of the present invention that water soluble peptides such as desmopressin may be formulated in solid dosage forms of the invention without the need for bonding to an ion exchange resin. Such dosage forms may therefore be free of an ion exchange resin. For hydrophobic peptides, which desmopressin is not, a surfactant may be present, as taught in U.S. Pat. No. 5,827,541, to which reference is made for further details. For peptides with an unpleasant taste (which desmopressin does not have), a lipid such as a lecithin may be present to improve patient acceptability, as taught in U.S. Pat. No. 6,156,339, to which reference is made for further details. Other strategies for taste masking include conversion of a soluble salt to a less soluble salt or to the free base, as taught by U.S. Pat. No. 5,738,875 and U.S. Pat. No. 5,837,287, and the use of a process disclosed in U.S. Pat. No. 5,976,577 wherein, prior to freeze drying, a suspension of uncoated or coated coarse particles of the pharmaceutically active substance(s) in a carrier material is cooled to reduce the viscosity and minimize release of the active substance during processing, as well as beyond the point of disintegration of the form in the mouth, to minimize bad taste from the peptide; reference is made to the cited patents for further details.

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For insoluble or poorly soluble peptides having a large particle size, xanthan gum may be present, particularly when the carrier is formed from gelatin, as the xanthan gum may act as a gelatin flocculating agent, as disclosed in U.S. Pat. No. 5,631,023, to which reference is made for further details.

As taught by WO-A-9323017 one or more amino acids having from about 2 to 12 carbon atoms may be present, when the matrix is selected from the group consisting of gelatin, pectin, soy fibre protein and mixtures thereof. In this formulation the preferred amino acid is glycine, while the preferred matrix forming agent is gelatin and/or pectin; in a particularly preferred embodiment, the dosage form additionally comprises mannitol. All excipients will be chosen to be pharmaceutically acceptable.

Pharmaceutical dosage forms of the present invention may be prepared by a process as described in GB-A-1548022, which comprises subliming solvent from a composition comprising the pharmaceutical substance and a solution of the carrier material in a solvent, the composition being in the solid state in a mould.

The sublimation is preferably carried out by freeze drying a composition comprising the active ingredient and a solution of the carrier material in a solvent. The composition may include additional ingredients, such as those mentioned above. The solvent is preferably water but it may contain a co-solvent (such as an alcohol e.g. tert-butyl alcohol) to improve the solubility of the chemical. The composition may also contain a surfactant e.g. Tween 80 (polyoxyethylene (20) sorbitan mono-oleate). The surfactant may help to prevent the freeze dried product sticking to the surface of the mould. It may also aid in the dispersion of the active ingredient.

The composition may contain a pH adjusting agent to adjust the pH of a solution from which the dosage form is prepared within the range of from 3 to 6, preferably from 3.5 to 5.5, and most preferably from 4 to 5, for example 4.5 or 4.8. Citric acid is a preferred pH adjusting agent, but others including hydrochloric acid, malic acid can be used. Such non-volatile pH adjusting agents will not be removed by the freeze drying or other sublimation process and so may be present in the final product.

The mould may comprise a series of cylindrical or other shape depressions in it, each of a size corresponding to the desired size of the shaped article. Alternatively, the size of the depression in the mould may be larger than the desired size of the article and after the contents have been freeze dried the product can be cut into the desired size (for example thin wafers).

However, as described in GB-A-2111423, the mould is preferably a depression in a sheet of filmic material. The filmic material may contain more than one depression. The filmic material may be similar to that employed in conventional blister packs which are used for packaging oral contraceptive tablets and like medicament forms. For example the filmic material may be made of thermoplastic material with the depressions formed by thermoforming. The preferred filmic material is a polyvinyl chloride film. Laminates of filmic material may also be used.

In one embodiment the mould comprises a metal plate (e.g. an aluminium plate) containing one or more depressions. In a preferred process using such a mould, the mould is cooled with a cooling medium (e.g. liquid nitrogen or solid carbon dioxide). When the mould is cooled a predetermined amount of water containing the carrier material, the active ingredient and any other desired ingredient is fed into the depression(s). When the contents of the depression(s) are frozen the mould is subjected to reduced pressure and, if desired, controlled application of heat to aid the sublimation. The pressure can be

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below about 4 mm. Hg; GB-A-1548022 teaches the employment of pressures of below 0.3 mm Hg, for example 0.1 to 0.2 mm is preferred. The freeze dried products may be removed from the depressions in the mould and stored for future use, e.g. in airtight jars or other suitable storage containers. Alternatively, the freeze dried product may be enclosed by filmic material as described in GB-A-2111423.

A later developed process useful for making pharmaceutical dosage forms in accordance with the invention is described in GB-A-2111423, to which reference is made for further details. The process comprises filling a composition comprising a predetermined amount of active ingredient and a solution of partially hydrolysed gelatin into a mould, freezing the composition in the mould by passing gaseous cooling medium over the mould and then subliming solvent from the frozen composition so as to produce a network of partially hydrolysed gelatin carrying the active ingredient.

In order to help ensure an even thickness of product, the side wall or walls of the mould may diverge outwards from the base and making an angle with the vertical of at least 5° at the surface of the composition, as described in GB-A-2119246 to which reference is made for further details.

Alternatively or in addition, pharmaceutical dosage forms of the present invention may be prepared by a process as described in GB-A-2114440 which comprises freezing a composition comprising a solution in a first solvent of a water-soluble or water dispersible carrier material that is inert towards the active ingredient, subliming the first solvent from the frozen composition so as to produce a product having a network of carrier material, adding to said product a solution or suspension of a second non-aqueous solvent containing a predetermined amount of the active ingredient and allowing or causing the second solvent to evaporate. Reference is made to GB-A-2114440 for further details.

Alternatively or in addition, pharmaceutical dosage forms of the present invention may be prepared by a process as described in GB-A-2111184, which comprises introducing the liquid medium in the form of droplets beneath the surface of a cooling liquid which is maintained at a temperature lower than the freezing point of the liquid medium, the cooling liquid being immiscible with, and inert with respect to, the liquid medium and having a density greater than that of both the liquid medium and the resulting frozen particles such as the liquid droplets float upwards in the cooling liquid towards the surface thereof, they are frozen to form spherical particles. The frozen spherical particles can be collected at or near the upper surface of the cooling liquid. Reference is made to GB-A-2111184 for further details.

Dosage forms in accordance with the invention have improved bioavailability. They are intended to be taken orally, and are highly suitable for that purpose. They disperse rapidly in the mouth, and may for example be placed under the tongue (sub-lingually).

According to a second aspect of the invention, there is provided a dosage form as described above for use in medicine, particularly, for voiding postponement, incontinence, primary nocturnal enuresis (PNE), nocturia and central diabetes insipidus.

The invention provides a method of postponing voiding, treating or preventing incontinence, primary nocturnal enuresis (PNE), nocturia and/or central diabetes insipidus, the method comprising administering an effective and generally non-toxic amount of desmopressin to a subject across the sublingual mucosa, for example in a dosage form as described above. Any other disease or condition treatable or preventable by desmopressin may similarly be addressed by means of invention. The invention therefore extends to the use of des-

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mopressin in the manufacture of a sublingually absorbable pharmaceutical formulation. The invention also extends to a pack comprising a sublingually absorbable pharmaceutical dosage form of desmopressin together with instructions to place the dosage form under a patient's tongue.

Encompassed within the invention is also a method for preparing a packaged dosage form of desmopressin, the method comprising bringing into association a sublingually absorbable pharmaceutical dosage form of desmopressin and instructions to place the dosage form under a patient's tongue. The instructions may for example be printed on packaging encompassing the dosage form when sold or dispensed, or may be on a product information leaflet or insert within the packaging.

Other peptides apart from desmopressin are formulatable in the formulations described above. The invention therefore extends to a pharmaceutical dosage form of a pharmaceutically active peptide adapted for oral absorption.

According to a further aspect of the invention, there is provided a solid pharmaceutical dosage form, for example for oral administration, the dosage form comprising a pharmaceutically active peptide and an open matrix network carrying the peptide, the open matrix network being comprised of a water-soluble or water-dispersible carrier material that is inert towards the peptide.

Although oral vaccines made from fast dissolving dosage forms are known from WO-A-9921579, there is no disclosure of pharmaceutically active peptides retaining their activity after administration. The experimental work in WO-A-9921579 merely shows the presence in saliva of IgA antibodies to tetanus toxoid following the administration of tetanus toxoid by means of an adjuvanted fast dissolving dosage vaccine formulation. Formulations of the present invention are not vaccines and do not include adjuvants.

Pharmaceutical dosage forms of this aspect of the invention contain a pharmaceutically active peptide. Such peptides may be directly active per se or they may have one or more active metabolites, i.e. they may be prodrugs for the primary or true active principle. The peptides may have for example from 2 to 20, preferably from 5 to 15, amino acid residues (at least some of which may be D-isomer, although L-isomers will generally be predominant). The peptides may be linear, branched or cyclic, and may include natural residues or substituents or residues or substituents not found in natural peptides or proteins either commonly or at all. Pharmaceutically acceptable salts, simple adducts and tautomers are included where appropriate.

Examples of peptides usefully formulated by means of the invention include somatostatin and its analogues including Cyclo(MeAla-Tyr-D-Trp-Lys-Val-Phe) and Cyclo(Asn-Phe-Phe-D-Trp-D-Lys-Thr-Phe-GABA), enkephalins including Met⁵-enkephalin and Leu⁵-enkephalin, oxytocin analogues such as atosiban (1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Om-oxytocin), GnRH analogues such as triptorelin (6-D-Trp-GnRH), leuprolide ([D-Leu⁶, Pro⁸-NHET]-GnRH), degarelix (Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(L-Hydroxyrotyl)-D-4Aph(Cbm)-Leu-Ilys -Pro-D-Ala-NH₂, where 2Nal is 2-naphthylalanine, 4Cpa is 4-chlorophenylalanine, 3Pal is 3-pyridylalanine, Ilys is N(8)-isopropyllysine, 4Aph is 4-aminophenylalanine and Cbm is the carbamoyl group) and other GnRH antagonists disclosed in U.S. Pat. No. 5,925,730 and U.S. Pat. No. 4,072,668, and vasopressin analogues such as desmopressin. It is particularly preferred to formulate by means of the invention agonists of naturally active peptides, such as those described above, since agonists may be active at lower doses than antagonists

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Dosage will be as determined by the physician or clinician, depending on the nature of the peptide, the nature of the disease or condition being treated or prevented, and other factors.

The invention extends to the use of a peptide in the manufacture of a dosage form as described above for treating or preventing a disease or condition which is treatable or preventable by a peptide.

The invention also provides a method of preventing a disease or condition which is treatable or preventable by a peptide, the method comprising administering an effective and generally non-toxic amount of the peptide to a subject in a dosage form as described above.

Low Dosage Analysis and Applications

As indicated above, doses and plasma/plasma/serum concentrations of desmopressin which are from 5 to 40% of the current recommended doses and resulting plasma/plasma/serum levels are therapeutically effective and in some cases safer for certain disease conditions such as CDI, PNE, and additional clinical indications requiring pharmacological concentration of the urine.

Clinical observations in adult males and females treated with desmopressin for a condition known as nocturia (which results in frequent night time urination) suggested that lower dosages of desmopressin would be desirable. In this patient population, standard intranasal and oral doses of desmopressin produced an unexpectedly high incidence of hyponatremia, a condition in which plasma/plasma/serum sodium falls to abnormally low levels. Hyponatremia can result in seizures, cardiac arrhythmias, cerebral edema and death. The oral doses of desmopressin were in the 100 to 400 mcg range and the intranasal doses were in the 10 to 20 mcg range. While these doses decreased the incidence of nocturia, the hyponatremia suggested that the doses were unnecessarily high resulting in an excessive duration of pharmacodynamic effect on urine concentration with consequent overhydration and dilutional lowering of plasma/plasma/serum sodium. Lower doses of desmopressin would produce adequate but not excessive antidiuresis in terms of the magnitude and duration of action.

In accordance with the present invention, plasma/plasma/serum desmopressin concentrations following administration of the pharmaceutical composition of the invention preferably range from about 0.1 pg/mL to about 10.0 pg/mL, and more preferably from about 0.5 pg/mL to about 5.0 pg/mL. These amounts and ranges of desmopressin may be administered by any method known in the art, including, without limitation, intravenous (bolus, infusion); subcutaneous (bolus, infusion, depot); intranasal; transmucosal (buccal and sublingual, e.g., orodispersible tablets, wafers, film, and effervescent formulations; conjunctival (eyedrops); rectal (suppository, enema)); transdermal (passive via patch, gel, cream, ointment or iontophoretic); or intradermal (bolus, infusion, depot) as outlined below. Additionally, pharmaceutical compositions that contain desmopressin in an amount that provide the above plasma/plasma/serum desmopressin levels may be prepared by the above methods and using the above carriers, or any other method known in the art.

The dose ranges of desmopressin outlined above can produce appropriate antidiuretic effect when administered by various routes as summarized in the examples below:

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| Route of Administration | Effective Daily Dose Range |
|---|----------------------------|
| Intravenous (bolus and infusion) | 0.5 ng-2000 ng |
| Subcutaneous (bolus, infusion, depot) | 0.5 ng-2000 ng |
| Intranasal | 0.1 mcg-20 mcg |
| Transmucosal including buccal and sublingual (orodispersible tablets, wafers, film and effervescent formulations), conjunctival (eyedrops), rectal (suppository, enema) | 0.1 mcg-20 mcg |
| Transdermal (passive via patch, gel, cream, ointment or iontophoretic) | 0.05 mcg-10 mcg |
| Intradermal (bolus, infusion, depot) | 0.05 mcg-10 mcg |

Administration of low dosages of desmopressin can be an effective treatment regimen for clinical indications such as treatment of central diabetes insipidus, prevention of primary nocturnal enuresis, prevention of nocturia, treatment of clinical disorders associated with nocturia including but not limited to sleep disturbances, prevention of incontinence (stress, urge, and the like), and voiding postponement during waking hours.

Specific formulations of desmopressin may also be created which enhance absorption and increase its systemic bioavailability. These formulations can result in incremental pharmacological effects at each point along the dose response curve, thus amplifying the activity of even low doses of desmopressin.

EXAMPLES

The present invention is further described in detail by means of the following Examples. All parts and percentages are by weight unless explicitly stated otherwise.

Example 1

200 µg Desmopressin Orodispersible Dosage Form

Spray-dried fish gelatin (4 g) and mannitol (3 g) are added to a glass beaker. Purified water (93 g) is then added and solution effected by stirring using a magnetic follower. The pH is checked and adjusted to 4.8 with citric acid as necessary. A Gilson pipette can then be used to deliver 500 mg of this solution into each one of a series of pre-formed blister pockets having a pocket diameter of about 16 mm. The blister laminate may comprise PVC coated with PVdC. The dosed units are then frozen at a temperature of -110° C. in a freeze tunnel with a residence time of 3.2 minutes and the frozen units are then held in an upright freezer for a time greater than 1.5 hours at a temperature of -25° C. (±5° C.). The units are then freeze-dried overnight with an initial shelf temperature of 10° C. rising to +20° C. at a pressure of 0.5 mbar. The units can be checked for moisture prior to unloading by the drying trace and by the pressurized moisture check.

In this way, following the general procedure given in Example 1 of WO-A-0061117, a desmopressin orodispersible dosage form is prepared using the following ingredients per unit dosage form:

| | |
|---|-----------------------------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 200 µg |
| Mannitol EP/USP (Roquette, Mannitol 35) | 15 mg |
| Fish gelatin USNF/EP | 20 mg |
| Citric acid (if necessary) (pH adjusting agent) | q.s. to pH 4.8 |
| Purified water | (Removed during processing) |

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Example

2 400 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 1 herein is followed, except that the amount of desmopressin per unit dosage form was 400 µg.

Example

3 800 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 1 herein is followed, except that the amount of desmopressin per unit dosage form was 800 µg.

Example 4

200 µg Desmopressin Orodispersible Dosage Form

Following the general procedure given in Example 1 of WO-A-0061117, a desmopressin dosage form orodispersible dosage form was prepared using the following ingredients per unit dosage form:

| | |
|---|-----------------------------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 200 µg |
| Mannitol EP/USP (Roquette, Mannitol 35) | 6 mg |
| Fish gelatin USNF/EP | 10 mg |
| Citric acid (if necessary) (pH adjusting agent) | q.s. to pH 4.8 |
| Purified water | (Removed during processing) |

Example 5

400 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 4 herein was followed, except that the amount of desmopressin per unit dosage form was 400 µg.

Example 6

800 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 4 herein was followed, except that the amount of desmopressin per unit dosage form was 800 µg.

Comparative Example 1

Desmopressin i.v. Solution

An injectable preparation of desmopressin was conventionally prepared using the following ingredients:

| | |
|--|--------------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 4 mg |
| Sodium chloride | 9 mg |
| (National Corporation of Swedish Pharmacies, Sweden) | |
| Hydrochloric acid (1N) (Merck, Germany) | q.s. to pH 4 |
| Water for injection | q.s. to 1 ml |

Comparative Example 2

200 µg Desmopressin Conventional Tablet

Using a conventional wet granulation process, tablets containing the following ingredients were prepared:

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| | |
|---|-----------------------------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 200 µg |
| Lactose (Pharmatose 150M, DMV, The Netherlands) | 120 mg |
| Potato starch (Lyckeby AB, Sweden) | 77 mg |
| PVP (Kollidon 25, BASF, Germany) | 1.8 mg |
| Magnesium stearate (Peter Greven, Germany) | 1 mg |
| Granulation Liquid (water, ethanol) | (Removed during processing) |

Comparative Example 3

100 µg Desmopressin Conventional Tablet

The procedure of Comparative Example 2 was followed, except that the amount of desmopressin was 100 µg per tablet.

Example 7

Bioavailability Of Desmopressin Administered in Accordance with Examples 4 to 6

Study Design

Twenty-four healthy non-smoking male volunteers were enrolled in the present study. The study was designed as a one-centre, open-labelled, randomized, balanced, 4-way cross-over phase I study. Each subject was, in a randomized order, administered sublingually desmopressin as a 200 µg, 400 µg and 800 µg orodispersible dosage form (Examples 4, 5 and 6, respectively) and 2 µg as an i.v. bolus dose (Comparative Example 1). Between the doses there was a washout period of 72 hours. In order to standardize the buccal mucosa before administration of the orodispersible tablet, the subjects were asked to avoid foods, chewing gum etc. Subjects were allowed to brush their teeth in the morning before dosing, but without toothpaste.

Blood Samples

Blood samples for plasma concentration of desmopressin were collected according to the following schedule: pre-dose and 15, 30 and 45 min and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours post-dosing. After intravenous administration additional blood samples were collected 5 and 10 minutes post-dosing.

Assay

The concentration of desmopressin in plasma was determined by a validated RIA method.

Pharmacokinetic Analysis

The concentration of desmopressin in plasma was analyzed for the individual volunteer in each administration group, by use of non-compartmental methods using the commercially available software WinNonlin™ Pro, ver. 3.2 (Pharsight Corporation, US). A plasma concentration value below limit of quantitation (LOQ) followed by values above LOQ was set at 'LOQ/2' for the NCA analysis and for the descriptive statistics on concentrations. Values below LOQ not followed by values above the LOQ are excluded from the NCA analysis, and set to zero in the descriptive statistics on concentrations.

Results of Pharmacokinetic Analysis

After i.v. administration the mean volume of distribution at steady state (V_{ss}) was 29.7 dm³. The mean clearance was calculated to be 8.5 dm³/hr and the mean elimination half-life was determined to be 2.8 hours. After oral administration of

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desmopressin maximum plasma concentrations were observed at 0.5-2.0 hours after dosing. The maximum plasma concentration was 14.25, 30.21 and 65.25 pg/ml after an oral dose of 200, 400 and 800 µg, respectively. After reaching the maximum value desmopressin was eliminated with a mean elimination half-life in the range of 2.8-3.0 hours. The bioavailability was determined to be 0.30% with at 95% confidence interval of 0.23-0.38%.

The pharmacokinetics of desmopressin is linear, when administered as the orodispersible dosage form of Example 4, 5 or 6.

Comparative Example 4

Bioavailability of Desmopressin Administered in Accordance with Comparative Examples 2 and 3

Thirty-six healthy male volunteers (Caucasian, Black and Hispanic) were enrolled in this study, which was designed as an open label, single dose, 3-way crossover study. Each subject was, in a randomized order, administered 200 µg desmopressin as a single 200 µg tablet (Comparative Example 2), 200 µg desmopressin as two 100 µg tablets (Comparative Example 3) and 2 µg as an i.v. bolus dose (Comparative Example 1).

After i.v. administration the mean elimination half-life was determined to be 2.24 hours. After oral administration of desmopressin maximum plasma concentrations were observed at 1.06 hours (2×100 µg) or 1.05 hours (1×200 µg) after dosing. The maximum plasma concentration was 13.2 and 15.0 pg/ml after an oral dose of 2×100 µg and 1×200 µg, respectively. The bioavailability was determined to be 0.13% (2×100 µg) or 0.16% (1×200 µg).

Example 8

Crossover Study Investigating the Antidiuretic Effect of Three Low Doses of Desmopressin

The following Example describes a study showing the antidiuretic effect of three low doses of desmopressin administered via intravenous infusion for 2 hours in over-hydrated, healthy, non-smoking male and female volunteers. Briefly, an open-label, crossover study with 8 healthy, over-hydrated, non-smoking male and female volunteers, age 18-40. The subjects were dosed initially with 0.5 ng/kg dose, then with the 1.0 ng/kg dose and finally the 2.0 ng/kg dose. Pharmacodynamic and pharmacokinetic parameters were evaluated at each dose level. A washout period of two days (48 hours) was observed between dosing.

Eight subjects evaluated in this study, 5 males, and 3 females. Their weights in kilograms were: 85.9, 65, 80.9, 63.3, 72.5, 67.6, 63.5, and 54.5. The mean weight of the 8 subjects was 69.15 kg, which is very close to the standard 70 kg weight estimate upon which the doses and blood levels of desmopressin in this study are based. Subjects were over-hydrated on study day 1 (first day of dosing) by drinking a volume of water equal to 1.5% of body weight and maintained by replacing urine output with water ingestion. Desmopressin of 0.5, 1.0 and 2.0 ng/kg in 100 mL of sterile, physiological saline (0.9%), USP for injection, was used in the study. Three infusions of desmopressin (one at each of the above concentrations) was administered as an I.V. infusion at a constant rate, each 2 hours in duration on days 1, 3 and 5 of the study. Each subject remained in the clinic from one day prior to first dosing to one day after last dosing for a total of 7 days. The first dose was 0.5 ng/kg. Following the end of the desmopressin infusion, subjects voided every 20 minutes and were

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monitored until 3 consecutive urine collections measured a urine output level exceeding 10 mL/min. At this point over-hydration was discontinued. Urine osmolality was measured 20 minutes before the infusion, at baseline, and with every 20 minute urine collection up to 6 hours after the start of the infusion. Urine-specific gravity was also measured. Plasma/serum sodium and plasma/serum osmolality was measured prior to dosing and at 2, 4, and 6 hours after the start of the infusion. Blood samples for pharmacokinetic determinations were collected predose, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after the start of the infusion. This same procedure was followed for the 1.0 ng/kg and 2.0 ng/kg infusions. On day 6, approximately 24 hours after the third and last desmopressin infusion subjects had an exit physical examination with vital signs, blood and urine laboratory assessments.

Criteria for evaluation in the study included urine output over time, urine osmolality over time, urine-specific gravity over time, and plasma/plasma/serum osmolality and sodium over time. Statistical analysis on the above criteria was performed. The statistical analysis is descriptive and all statistical hypothesis testing was done for exploratory purposes. The following was investigated: duration of action, i.e., time from 'onset' to 'end' action was estimated for each subject using three different levels of osmolality as cut off (150 mOsm/kg, 200 mOsm/kg and 400 mOsm/kg). First, duration of action was defined as the time from onset of action (i.e., the first time after dose administration where urine osmolality was less than 150 mOsm/kg) to end of action (the first subsequent time where urine osmolality was less than 150 mOsm/kg and confirmed at the next interval unless the first subsequent time was the last observation point). The second and third estimation used 200 mOsm/kg and 400 mOsm/kg as cut off levels for 'onset' and 'end' of action, respectively. Subjects with no 'end' of action, with respect to the definition were censored at the time their urinary output returns to baseline (exceeds 10 mL/min) and/or the time where the over-hydration procedure stopped. The overall duration of action was estimated for each dose group using the nonparametric Kaplan-Meier method. The different approaches for estimating duration of action were expected to give lower and upper limits of the true

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probability, i.e., probability of desmopressin activity as a function of time. Furthermore, the duration of action was presented for each treatment group using the mean, SD, median, minimum and maximum values. The dose-response relationship between duration of action and dose was investigated using an appropriate linear or nonlinear model. Pharmacokinetic parameters were derived from the individual concentration versus time curves of desmopressin, i.e., AUC (area under the plasma concentration time curve to infinity), C_{max} (maximum plasma concentration observed), t_{max} (time of C_{max} after dosing), CL (total systemic clearance), V_z (volume of distribution during the terminal phase), AUC_t (area under the plasma concentration time curve from time zero to time t), λ_z (first order rate constant associated with the terminal (log-linear) portion of the plasma concentration time curve estimated via linear regression of the time vs. log of concentration) and $t_{1/2}$ (terminal half life).

Summary of Results:

All three doses (I.V. infusions) of desmopressin produced a measurable, antidiuretic effects in terms of increased urine concentration (osmolality) and decreased urine output in a dose response fashion. The pharmacodynamic duration of antidiuretic effect also demonstrated a dose response curve with the lowest dose having the shortest duration of effect. The mean peak urine osmolality (mOsm/kg) occurred at the end of the 2 hour infusion for each dose level. Baseline mean urine osmolality was 55.8, 55.8 and 55.6 mOsm/kg for 0.5, 1.0, 2.0 ng/kg doses, respectively. Mean peak urine osmolality was 206.0, 444.7 and 587.2 mOsm/kg at 2 hours for the 0.5, 1.0 and 2.0 ng/kg doses, respectively. The mean nadir urine output (mL/min) also occurred at the end of the 2 hour infusion for each dose level. Baseline mean urine output was 18.6, 16.6 and 16.9 mL/min for the 0.5, 1.0 and 2.0 ng/kg doses, respectively. Mean nadir urine output was 7.1, 1.3, and 0.7 mL/min for the 0.5, 1.0 and 2.0 ng/kg doses, respectively. The duration of antidiuretic effect was approximately 180 minutes for the 0.5 ng/kg dose, 240 to 280 minutes for the 1.0 ng/kg dose and 360 minutes for the 2.0 ng/kg dose. The urine osmolality and output results for each subject and the means for each time period are described in Tables 1-6 and FIGS. 1-9.

TABLE 1

| Sub- ject | Urine Osmolality (0.5 ng/kg) | | | | | | | | | | | | | | | | | | | |
|--------------|------------------------------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 |
| 01-001 | 61 | 61 | 63 | 75 | 84 | 91 | 100 | 104 | 93 | 57 | 78 | * | * | * | * | * | * | * | * | * |
| 01-002 | 41 | 43 | 46 | 55 | 70 | 83 | 91 | 90 | 79 | 72 | 66 | * | * | * | * | * | * | * | * | * |
| 01-003 | 57 | 57 | 65 | 105 | 162 | 228 | 338 | 447 | 363 | 243 | 177 | 122 | 103 | 93 | 80 | 88 | * | * | * | * |
| 01-004 | 49 | 49 | 97 | 100 | 57 | 56 | 58 | 61 | 59 | 57 | 55 | * | * | * | * | * | * | * | * | * |
| 01-005 | 57 | 60 | 95 | 110 | 89 | 83 | 84 | 87 | 80 | 74 | 71 | * | * | * | * | * | * | * | * | * |
| 01-006 | 80 | 85 | 115 | 294 | 476 | 621 | 633 | 655 | 670 | 601 | 521 | 390 | 327 | 274 | 215 | 250 | 193 | 156 | 133 | 120 |
| 01-007 | 52 | 54 | 56 | 72 | 86 | 95 | 108 | 119 | 87 | 75 | 65 | 59 | 57 | * | * | * | * | * | * | * |
| 01-008 | 49 | 52 | 48 | 55 | 65 | 69 | 78 | 85 | 75 | 67 | 61 | 60 | 59 | 58 | * | * | * | * | * | * |
| Mean | 55.8 | 57.6 | 73.1 | 108.3 | 136.1 | 165.8 | 186.3 | 206.0 | 188.3 | 155.8 | 136.8 | 157.8 | 136.5 | 141.7 | 147.5 | 169.0 | 193.0 | 156.0 | 133.0 | 120.0 |

TABLE 2

| Sub- ject | Urine Osmolality (1.0 ng/kg) | | | | | | | | | | | | | | | | | | | |
|--------------|------------------------------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 58 | 59 | 65 | 108 | 281 | 305 | 480 | * | 435 | 132 | 150 | 160 | 71 | 60 | * | * | * | * | * | * |
| 01-002 | 46 | 44 | 53 | 91 | 168 | 222 | 315 | 414 | 324 | 230 | 171 | 127 | 116 | 104 | 99 | 98 | 82 | 68 | 62 | * |
| 01-003 | 48 | 51 | 60 | 178 | 406 | 402 | 506 | 595 | 618 | * | 588 | 374 | 322 | 221 | 162 | 148 | 111 | 96 | * | * |
| 01-004 | 48 | 49 | 52 | 68 | 92 | 135 | 180 | 219 | 156 | 105 | 85 | 71 | 71 | 67 | * | * | * | * | * | * |
| 01-005 | 68 | 68 | 73 | 106 | 166 | 235 | 260 | 312 | 204 | 142 | 109 | 94 | 88 | 83 | 75 | * | * | * | * | * |
| 01-006 | 82 | 82 | 124 | 585 | 614 | 638 | 708 | 747 | 736 | 733 | 771 | 694 | * | 747 | 606 | 655 | 687 | 546 | 458 | 374 |
| 01-007 | 47 | 47 | 53 | 100 | 175 | * | 267 | 381 | * | 228 | 122 | 96 | 86 | 81 | 69 | 69 | 57 | 53 | 47 | 44 |
| 01-008 | 49 | 52 | 57 | 100 | 173 | * | 288 | * | * | * | 251 | 114 | 96 | 90 | 80 | * | 73 | 61 | 55 | 51 |
| Mean | 55.8 | 56.5 | 67.1 | 167.0 | 259.4 | 322.8 | 375.5 | 444.7 | 412.2 | 261.7 | 280.9 | 216.3 | 121.4 | 181.6 | 181.8 | 242.5 | 202.0 | 164.8 | 155.5 | 156.3 |

TABLE 3

| Sub- ject | Urine Osmolality (2.0 ng/kg) | | | | | | | | | | | | | | | | | | | |
|--------------|------------------------------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 63 | 63 | 88 | 373 | * | 526 | * | * | 585 | * | 571 | 623 | 482 | * | 458 | 384 | 346 | 146 | 73 | 76 |
| 01-002 | 40 | 40 | 46 | 149 | 251 | * | 492 | * | 601 | 533 | 538 | 489 | 385 | 348 | 252 | 244 | 173 | 116 | 87 | 76 |
| 01-003 | 51 | 52 | 73 | 337 | 401 | * | * | 568 | * | * | 568 | * | 541 | 559 | 477 | 476 | 380 | 267 | 179 | 134 |
| 01-004 | 45 | 48 | 50 | 146 | 298 | 390 | 442 | 461 | 478 | 439 | 357 | 250 | 195 | 139 | 110 | 112 | 97 | 73 | 66 | 60 |
| 01-005 | 78 | 73 | 119 | 293 | 499 | 501 | 421 | 564 | 492 | 492 | 390 | 387 | 352 | 267 | 195 | 178 | 154 | 104 | 98 | 87 |
| 01-006 | 71 | 73 | 108 | 604 | 626 | 698 | 748 | 769 | 771 | 727 | 733 | 676 | 677 | 668 | 640 | 665 | 648 | 585 | 577 | 547 |
| 01-007 | 45 | 45 | 60 | * | * | * | * | * | * | 509 | * | * | * | 666 | * | * | * | 255 | 100 | 79 |
| 01-008 | 52 | 54 | 61 | 208 | 385 | 465 | 525 | 574 | 533 | 508 | 583 | 542 | * | 539 | * | * | 473 | * | 204 | 91 |
| Mean | 55.6 | 56.0 | 75.6 | 301.4 | 410.0 | 516.0 | 525.6 | 587.2 | 576.7 | 534.7 | 534.3 | 494.5 | 438.7 | 455.1 | 355.3 | 343.2 | 324.4 | 220.9 | 173.0 | 143.8 |

TABLE 4

| Sub- ject | Urine Output (0.5 ng/kg) | | | | | | | | | | | | | | | | | | | |
|--------------|--------------------------|------|------|------|------|------|------|------|------|------|------|-----|-----|------|------|-----|-----|-----|-----|-----|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 20 | 18.8 | 16.7 | 14 | 10.9 | 10 | 9.1 | 5 | 17.6 | 15 | 13.6 | * | * | * | * | * | * | * | * | * |
| 01-002 | 17 | 16.5 | 16.4 | 13.8 | 10.8 | 9.3 | 8.8 | 8.3 | 11.8 | 10.5 | 13.6 | * | * | * | * | * | * | * | * | * |
| 01-003 | 18.8 | 17.4 | 16.7 | 9 | 5.9 | 4.1 | 2.4 | 2 | 2 | 3 | 6.4 | 8.3 | 8.5 | 11.4 | 15.6 | 15 | * | * | * | * |
| 01-004 | 26 | 22.3 | 8.9 | 10.5 | 16.4 | 16.5 | 16.5 | 15.2 | 16 | 17 | 17.1 | * | * | * | * | * | * | * | * | * |
| 01-005 | 19.5 | 20 | 11.8 | 9 | 12.5 | 10 | 9.5 | 10.9 | 12.5 | 13 | 15.7 | * | * | * | * | * | * | * | * | * |
| 01-006 | 15.9 | 13 | 8.8 | 3.1 | 1.4 | 1.1 | 1.1 | 0.9 | 1.2 | 1.8 | 2 | 2.2 | 3.8 | 4.2 | 3.8 | 3.2 | 4.1 | 5 | 6.4 | 7.3 |

TABLE 4-continued

| Sub- ject | <u>Urine Output (0.5 ng/kg)</u> | | | | | | | | | | | | | | | | | | | |
|--------------|---------------------------------|------|------|------|-----|-----|-----|-----|-----|-----|------|------|------|------|-----|-----|-----|-----|-----|-----|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 |
| 01-007 | 16.1 | 25.8 | 14 | 13.3 | 9.6 | 9.2 | 7 | 8 | 8.7 | 10 | 12.2 | 17.9 | 16.7 | * | * | * | * | * | * | * |
| 01-008 | 15.5 | 13.3 | 12.2 | 10 | 9.1 | 8.1 | 7.5 | 6.5 | 7.1 | 7.6 | 8.2 | 13.3 | 12.5 | 10.5 | * | * | * | * | * | * |
| Mean | 18.6 | 18.4 | 13.2 | 10.3 | 9.6 | 8.5 | 7.7 | 7.1 | 9.6 | 9.7 | 11.1 | 10.4 | 10.4 | 8.7 | 9.7 | 9.1 | 4.1 | 5.0 | 6.4 | 7.3 |

TABLE 5

| Sub- ject | Urine Output (1.0 ng/kg) | | | | | | | | | | | | | | | | | | | |
|--------------|--------------------------|------|------|------|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|-----|------|------|------|------|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 |
| 01-001 | 16.8 | 17.4 | 10.4 | 7.6 | 1.9 | 2.4 | 1.1 | 0 | 0.8 | 2.4 | 2.6 | 13.2 | 15.9 | 12.9 | * | * | * | * | * | * |
| 01-002 | 17.1 | 18 | 15.6 | 8.4 | 4.2 | 3.5 | 2.2 | 1.6 | 2.6 | 3.1 | 4.5 | 6 | 5.6 | 6.3 | 7.5 | 7.7 | 10.5 | 10.1 | 14.2 | |
| 01-003 | 18.5 | 18 | 14 | 4 | 1.4 | 1.6 | 0.9 | 0.7 | 0.8 | 0 | 1.7 | 1.6 | 2.1 | 3.9 | 5.8 | 5.7 | 8.8 | 10.6 | 13.9 | 15.5 |
| 01-004 | 22 | 19.3 | 17.1 | 12.5 | 8.5 | 4.8 | 3.7 | 3.2 | 5 | 8.1 | 10 | 12.4 | 11.6 | 14.1 | * | * | * | * | * | * |
| 01-005 | 19.5 | 20 | 15.2 | 9.9 | 5.7 | 3 | 3 | 2.6 | 4.3 | 5.3 | 7.9 | 8.8 | 11.8 | 11.8 | 11.7 | * | * | * | * | * |
| 01-006 | 13 | 12.4 | 7.2 | 1.2 | 0.8 | 0.6 | 0.7 | 0.6 | 0.7 | 0.7 | 0.7 | 0.6 | 0 | 1.1 | 0.8 | 0.6 | 0.9 | 1.1 | 1 | 1.9 |
| 01-007 | 16 | 15.9 | 13.2 | 6.5 | 3.7 | 0 | 4.3 | 1.3 | 0 | 5.8 | 5.1 | 6.9 | 7.3 | 7.7 | 9 | 9.3 | 8.9 | 11.6 | 16 | 15.7 |
| 01-008 | 10.2 | 12.5 | 11.2 | 5.7 | 3.5 | 0 | 3.7 | 0 | 0 | 0 | 7.1 | 4.3 | 4.2 | 5.5 | 4.7 | 0 | 11.5 | 6.7 | 8 | 8.6 |
| Mean | 16.6 | 16.7 | 13.0 | 7.0 | 3.7 | 2.0 | 2.5 | 1.3 | 1.8 | 3.2 | 5.0 | 6.7 | 7.3 | 7.9 | 6.6 | 4.7 | 8.1 | 8.0 | 10.6 | 10.4 |

TABLE 6

| Sub- ject | <u>Urine Output (2.0 ng/kg)</u> | | | | | | | | | | | | | | | | | | | |
|--------------|---------------------------------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | # | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 |
| 01-001 | 14.5 | 16 | 9.3 | 1.2 | 0 | 1.5 | 0 | 0 | 0.9 | 0 | 1 | 1.9 | 0.5 | 2.7 | 1.9 | 1.6 | 2.1 | 13.6 | 11.2 | 11.4 |
| 01-002 | 19.5 | 20 | 14.4 | 3 | 2.7 | 0 | 2.3 | 0 | 3.3 | 0.9 | 0.8 | 0.9 | 1.1 | 1.8 | 2.8 | 2.6 | 3.9 | 6.2 | 9.5 | 12.9 |
| 01-003 | 18.5 | 18.3 | 10.8 | 1.6 | 1.8 | 0 | 0 | 2.7 | 0 | 0 | 2.8 | 0 | 2.4 | 1.4 | 2 | 1.9 | 2 | 4.6 | 6.9 | 8.6 |
| 01-004 | 22 | 20.5 | 14.4 | 5.1 | 1.8 | 1.5 | 0.8 | 1 | 1.3 | 1.5 | 2 | 3.1 | 4.1 | 6 | 8.3 | 8.4 | 9.4 | 11.6 | 14.3 | 13.9 |
| 01-005 | 18 | 17.6 | 9.2 | 3.5 | 1.7 | 1.4 | 1.4 | 1 | 1.2 | 1.2 | 1.6 | 1.6 | 1.7 | 2.6 | 3.8 | 4.9 | 5.7 | 8.3 | 10.3 | 12.5 |
| 01-006 | 14 | 12.9 | 6.5 | 0.8 | 0.7 | 0.4 | 0.5 | 0.4 | 0.7 | 0.5 | 0.7 | 0.6 | 0.7 | 0.7 | 0.7 | 0.7 | 1 | 0.9 | 1 | 1.2 |
| 01-007 | 14.5 | 13.2 | 9.1 | 0 | 0 | 0 | 0 | 0 | 0 | 4.6 | 0 | 0 | 0 | 1.7 | 0 | 0 | 0 | 7.3 | 6.5 | 9.1 |
| 01-008 | 14.5 | 13.1 | 10 | 2.9 | 1.6 | 0.5 | 0.9 | 0.4 | 0.4 | 0.5 | 0.4 | 0.4 | 0 | 1 | 0 | 0 | 1.4 | 0 | 3.5 | 4.6 |
| Mean | 16.9 | 16.5 | 10.5 | 2.3 | 1.3 | 0.7 | 0.7 | 0.7 | 1.0 | 1.2 | 1.2 | 1.1 | 1.3 | 2.2 | 2.4 | 2.5 | 3.2 | 6.6 | 7.9 | 9.3 |

As shown in Tables 1-6 and FIGS. 1-9, low doses of desmopressin administered as I.V. infusions over 2 hours produced significant antidiuretic effects in over-hydrated, normal subjects in a dose response fashion. These doses and calculated plasma/serum concentrations of desmopressin

were far lower than the current labeled recommendations and current clinical practice by a factor of more than one order of magnitude. The pharmacodynamic duration of action was also proportional to the dose with the 1.0 and 2.0 ng/kg doses providing durations of 4 to 6 hours. This may be adequate to

produce the desired therapeutic effects for existing and potential new clinical indications for desmopressin. Safety and tolerability were excellent.

The results of this study confirm the low-dose hypothesis for desmopressin and provide an empirical basis for further clinical studies in patients to evaluate low doses of desmopressin for such conditions as primary nocturnal enuresis, adult nocturia, incontinence and central diabetes insipidus.

The therapeutic effectiveness of desmopressin for all these clinical indications is based on desmopressin's antidiuretic pharmacological effect which results in production of smaller volumes of more concentrated urine. For patients with central diabetes insipidus, the pituitary gland produces little or no vasopressin, the natural antidiuretic hormone. This deficiency results in large volumes of very dilute urine being produced which can lead to dehydration and serious metabolic abnormalities unless the patient consumes very large volumes of water. Desmopressin replaces the deficient vasopressin and restores normal urine concentration and volume in these patients. In patients with primary nocturnal enuresis (bed wetting), the antidiuretic effect of desmopressin decreases urine volume at night, lowering the amount of urine which the urinary bladder must retain and, thereby decreasing or eliminating occurrences of enuresis.

In patients with adult nocturia, there is either polycoma (production of large amounts of urine) at night, low bladder capacity or increased bladder sensitivity to urine volume. Under all these circumstances, the bladder's threshold for urine retention is exceeded during the night, often several times, resulting in neurological signals for voiding. This awakens the patient in order to void. Desmopressin's antidiuretic effect decreases urine production at night delaying the time when the voiding threshold is exceeded resulting in a longer sleep period before voiding and decreasing the number of nocturnal voids.

In patients with incontinence of various types (stress, urge, etc.) often related to urinary bladder abnormalities from surgery, childbirth, and aging, the bladder is unable to retain even normal volumes of urine. The volume threshold for voiding is low and there is a high risk of involuntary voiding (incontinence). Desmopressin's antidiuretic effect decreases urine production allowing for voiding postponement because there is a delay in crossing the abnormally low volume threshold for voiding in these patients.

In all the above clinical indications, or medical uses of desmopressin, its antidiuretic pharmacological effect resulting in decreased production of more concentrated urine is the mechanism of therapeutic effectiveness. This clinical study demonstrates that desmopressin can produce this essential antidiuretic effect at much lower doses and lower blood concentrations than previously thought. Therefore, lower doses and concentrations of desmopressin may be used for treating patients with all of the above conditions.

While the invention has been described above with reference to specific embodiments thereof, it is apparent that many changes, modifications, and variations can be made without departing from the inventive concept disclosed herein.

Accordingly, it is intended to embrace all such changes, modifications, and variations that fall within the spirit and broad scope of the appended claims. All patent applications, patents, and other publications cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method of treating nocturia, primary nocturnal enuresis, or incontinence, or for inducing voiding postponement, said method comprising administering to a patient in need thereof a pharmaceutical composition comprising a dose of desmopressin sufficient to achieve a maximum desmopressin plasma/serum concentration no greater than 10 pg/ml and maintaining the concentration within the range of about 0.5 pg/ml and 10 pg/ml for about four to six hours.

2. The method of claim 1, comprising administering said composition by transmucosal, transdermal, or intradermal delivery.

3. The method of claim 1, comprising treating nocturia.

4. The method of claim 1, comprising administering said composition by intravenous delivery.

5. The method of claim 1, comprising administering said composition by subcutaneous delivery.

6. The method of claim 1, comprising administering said composition by transmucosal delivery.

7. The method of claim 1, comprising administering said composition by transdermal delivery.

8. The method of claim 1, comprising administering said composition by intradermal delivery.

9. The method of claim 1, wherein the desmopressin plasma/serum concentration is maintained at a level no greater than about 5 pg/ml.

10. A method for inducing an antidiuretic effect in a patient comprising the step of administering to a patient a pharmaceutical composition comprising desmopressin by transmucosal, transdermal, or intradermal delivery in an amount and for a time sufficient to establish a maximum serum/plasma desmopressin concentration no greater than 10 pg/ml.

11. The method of claim 10, wherein said patient is suffering from incontinence, primary nocturnal enuresis (PNE), or nocturia.

12. The method of claim 10, wherein said desmopressin pharmaceutical composition is administered in an amount and for a time sufficient to establish a serum/plasma desmopressin concentration no greater than about 5 pg/ml.

13. A method for treating a patient suffering from nocturia comprising administering to a patient a pharmaceutical composition comprising desmopressin by transmucosal, transdermal, or intradermal delivery in an amount and for a time sufficient to establish a maximum serum/plasma desmopressin concentration greater than 0.1 pg/ml and less than 10 pg/ml.

14. The method of claim 10, wherein the patient is a human or other mammalian subject.

15. The method of claim 13, wherein said concentration is maintained greater than 0.1 pg/ml for a time greater than 4 hours.

* * * * *



US007579321B2

(12) **United States Patent**
Fein

(10) **Patent No.:** **US 7,579,321 B2**
(45) **Date of Patent:** ***Aug. 25, 2009**

(54) **PHARMACEUTICAL COMPOSITIONS
INCLUDING LOW DOSAGES OF
DESMOPRESSIN**

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See application file for complete search history.

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(57) **ABSTRACT**

The present invention is directed to a pharmaceutical composition comprising 0.5 ng to 20 µg desmopressin and a pharmaceutically acceptable carrier. The present invention is also directed to a pharmaceutical composition comprising desmopressin and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is effective to establish a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/serum to about 10.0 picogram desmopressin per mL plasma/serum. Articles of manufacture and methods of using the above invention are also disclosed.

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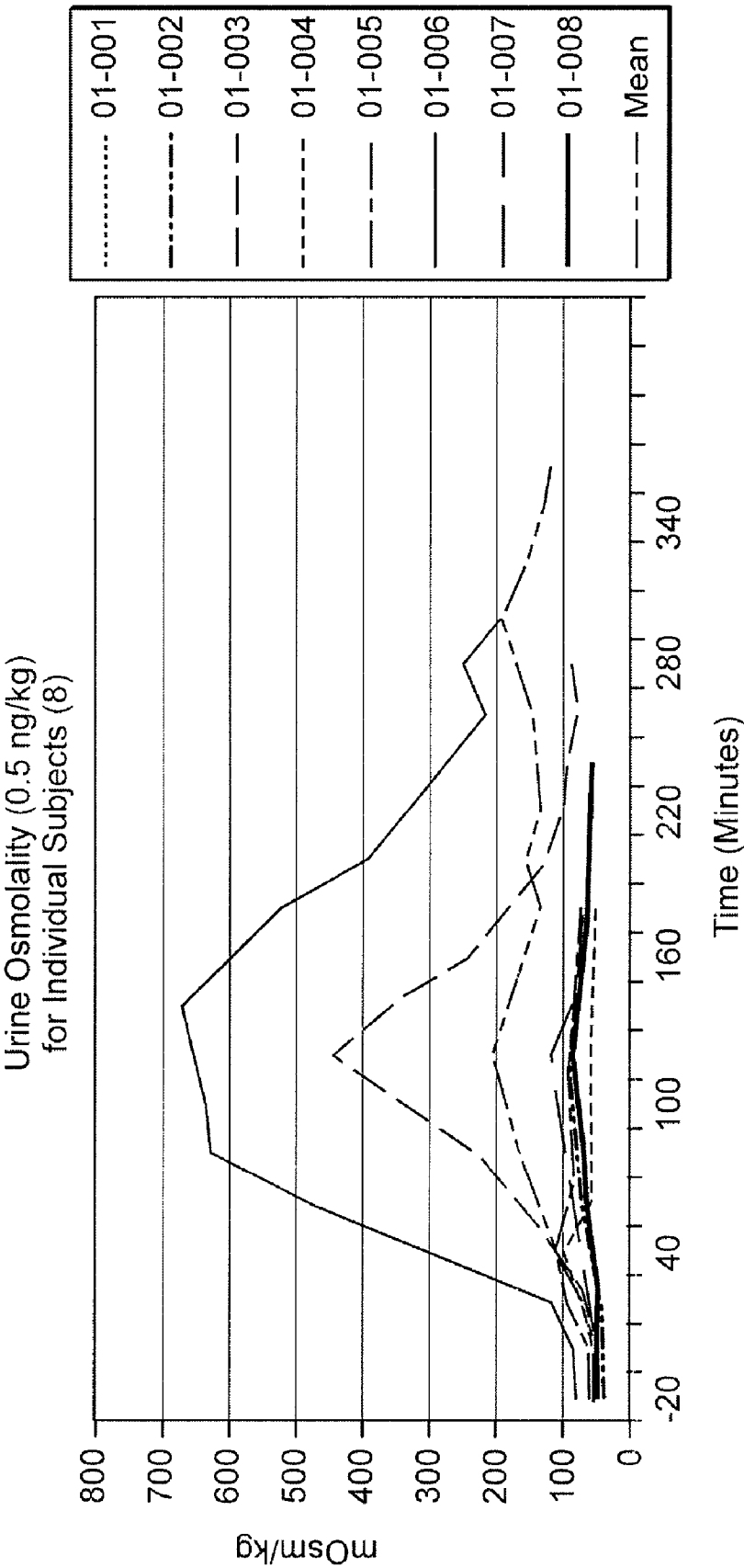


FIG. 1

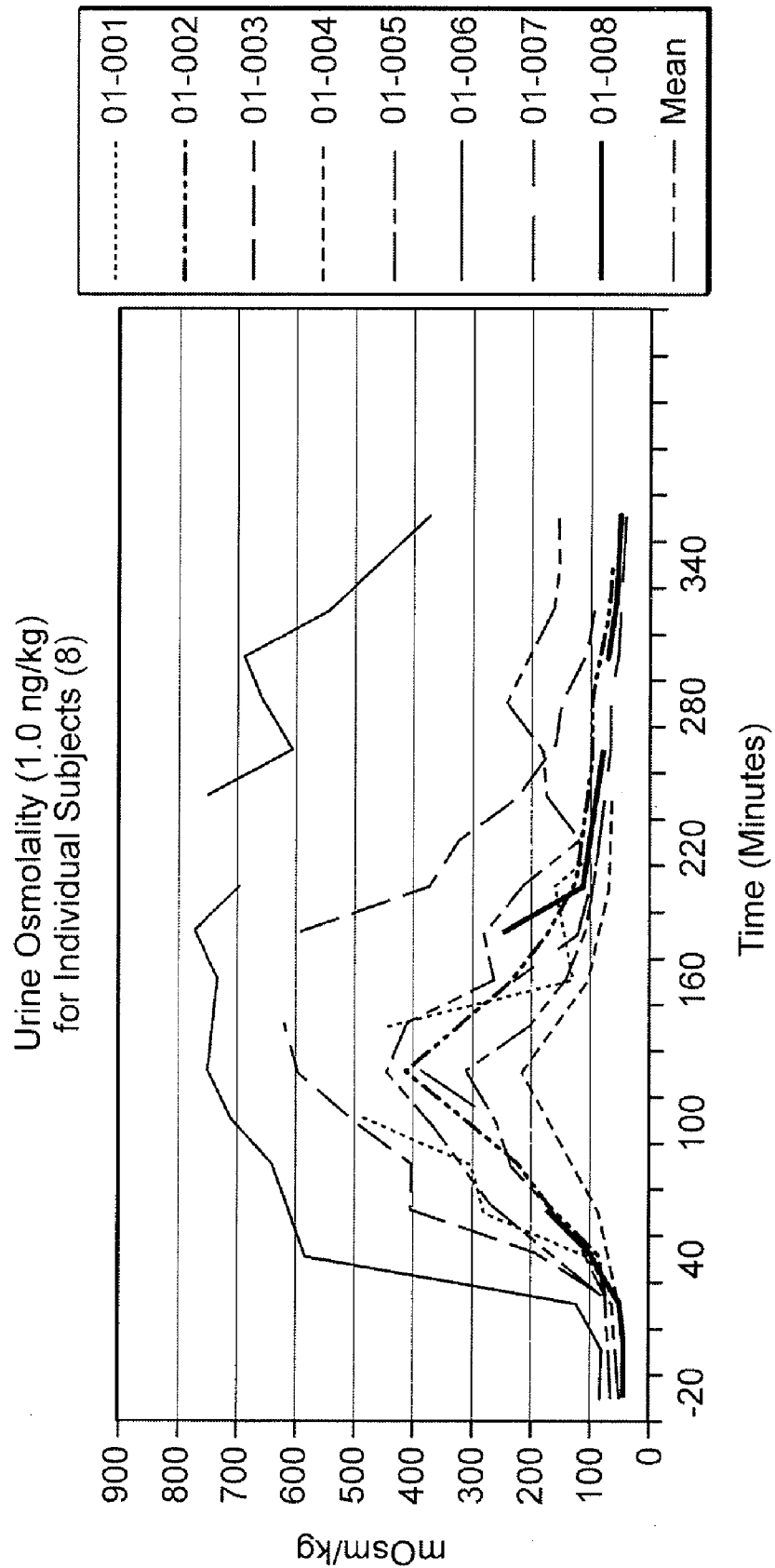


FIG. 2

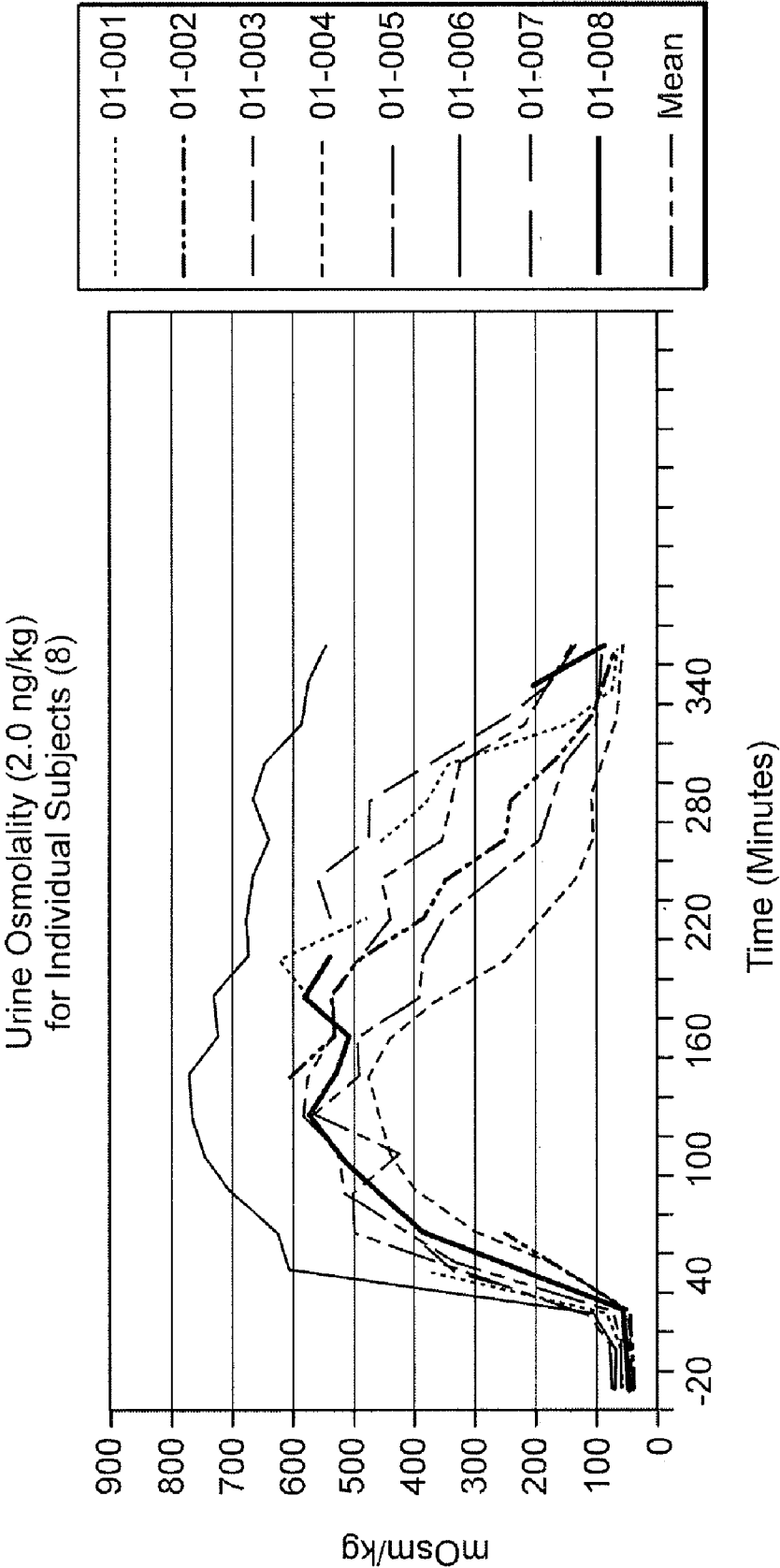


FIG. 3

Urine Output (0.5 ng/kg)
for Individual Subjects (8)

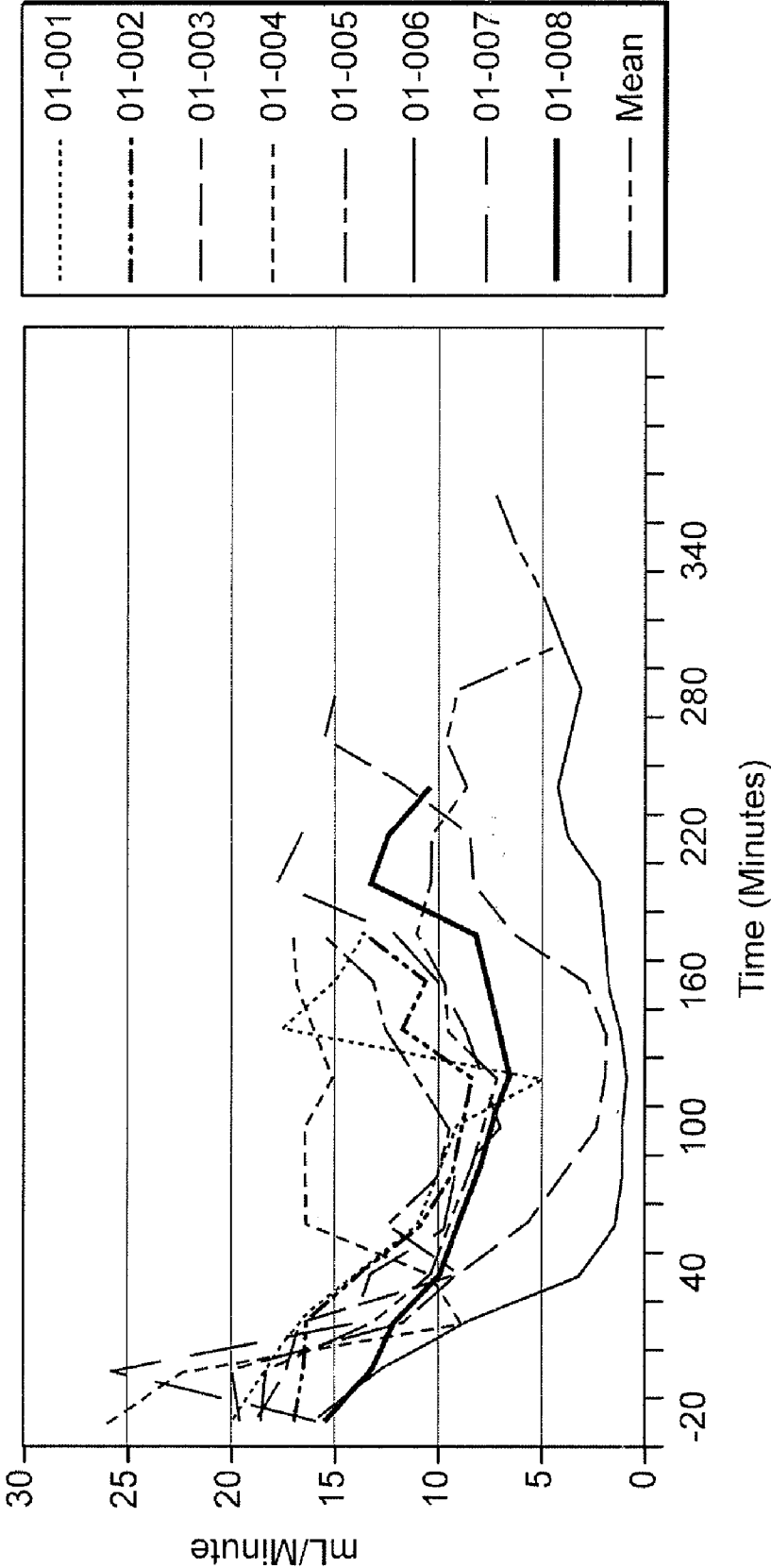


FIG. 4

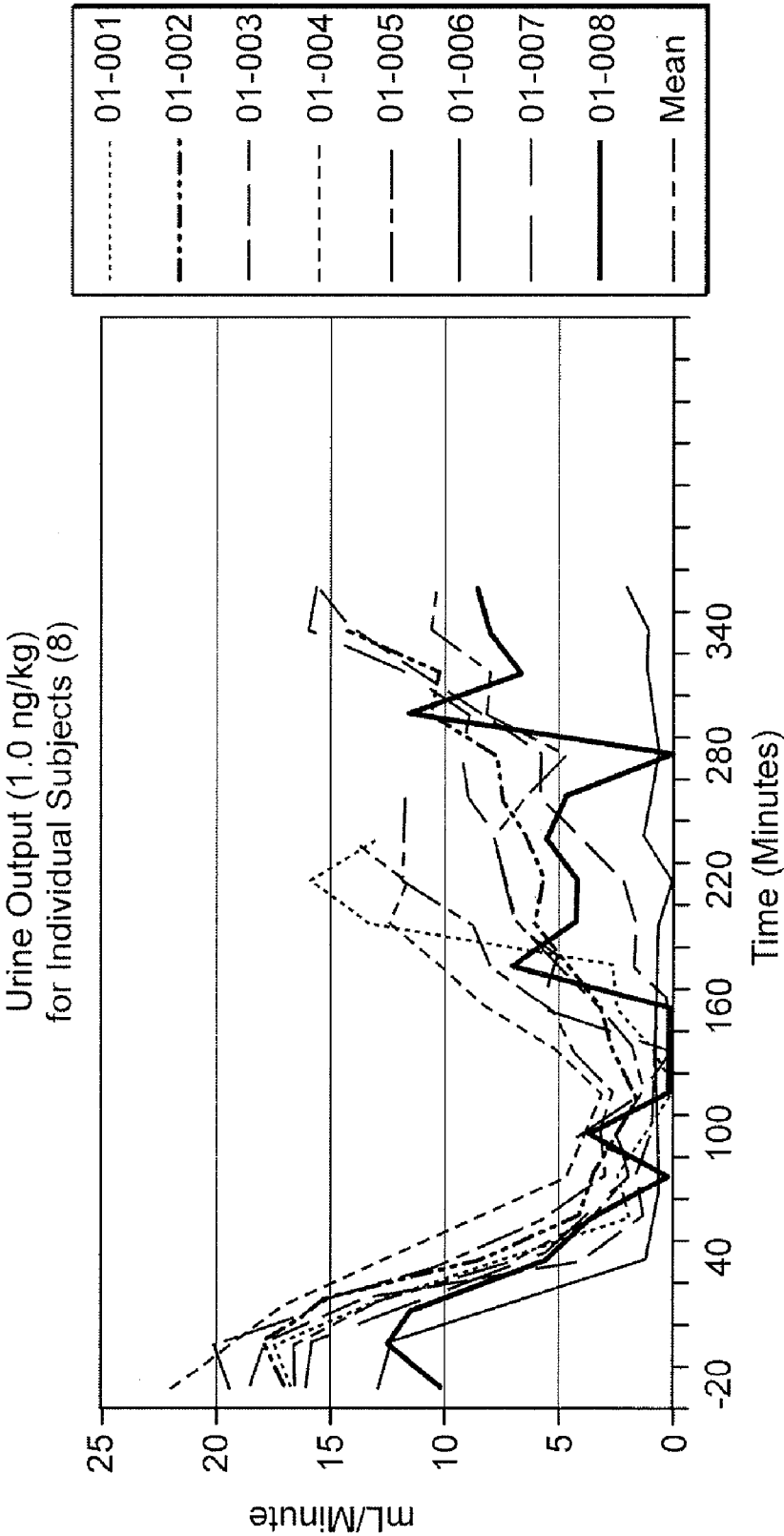


FIG. 5

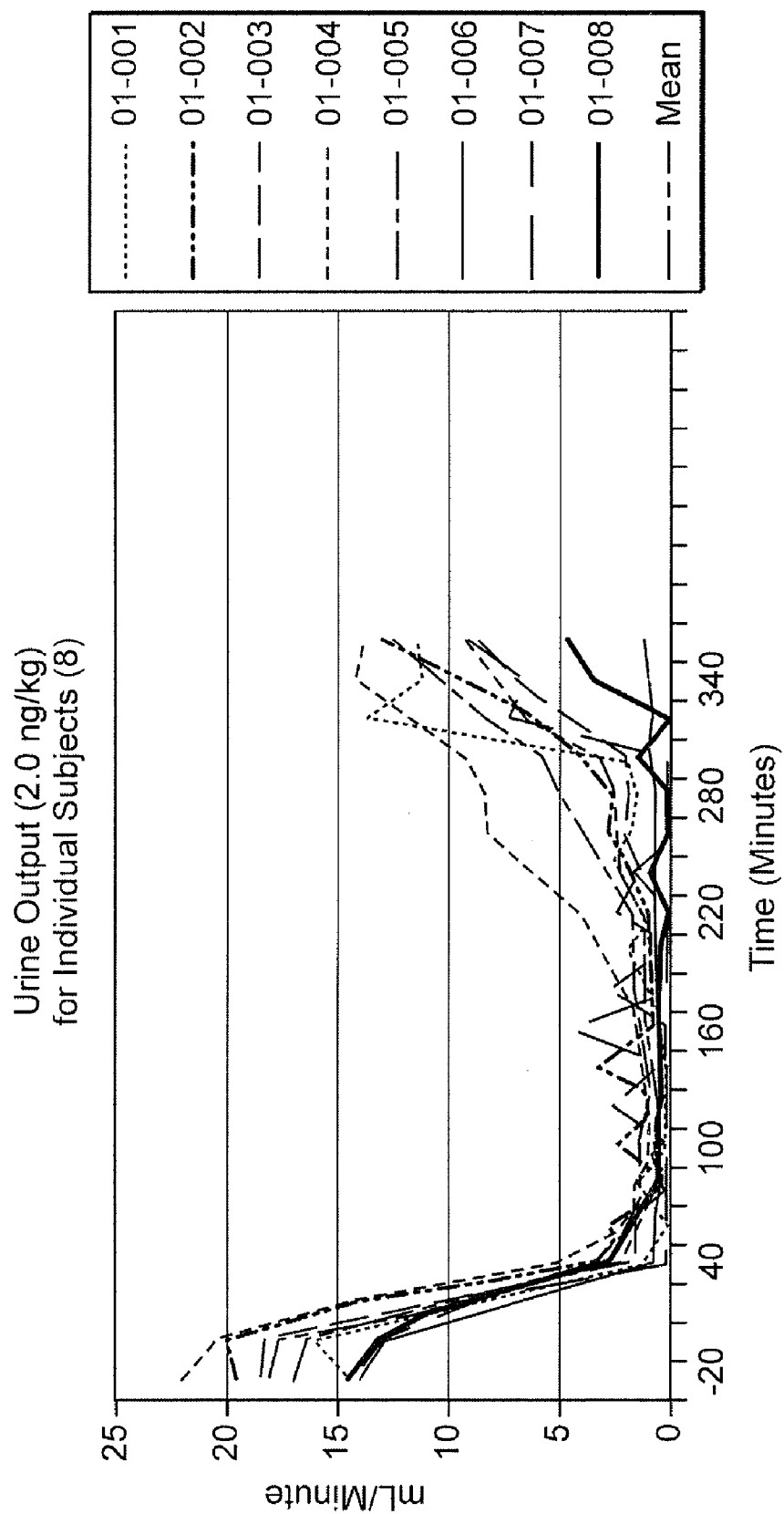


FIG. 6

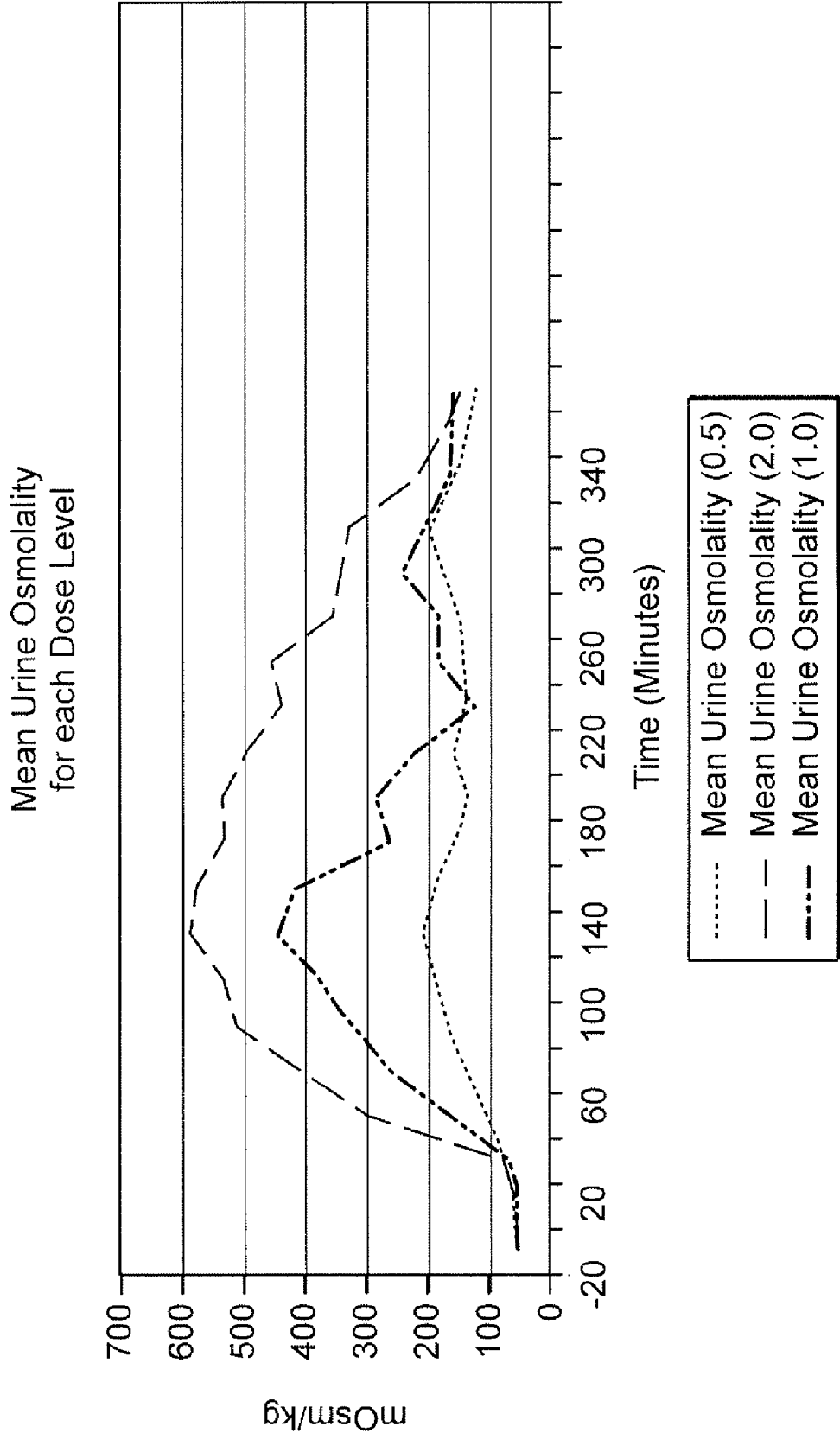


FIG. 7

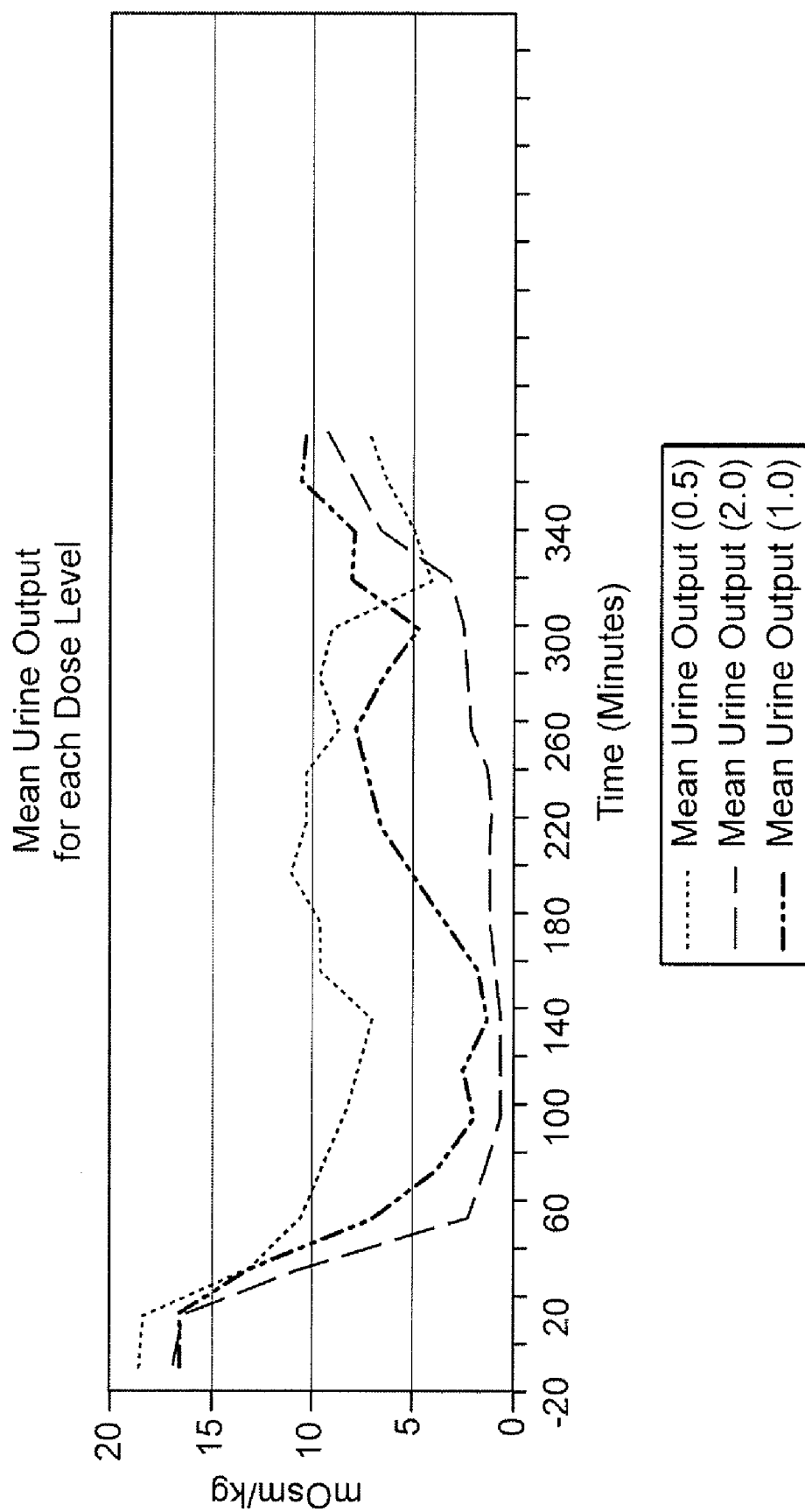


FIG. 8

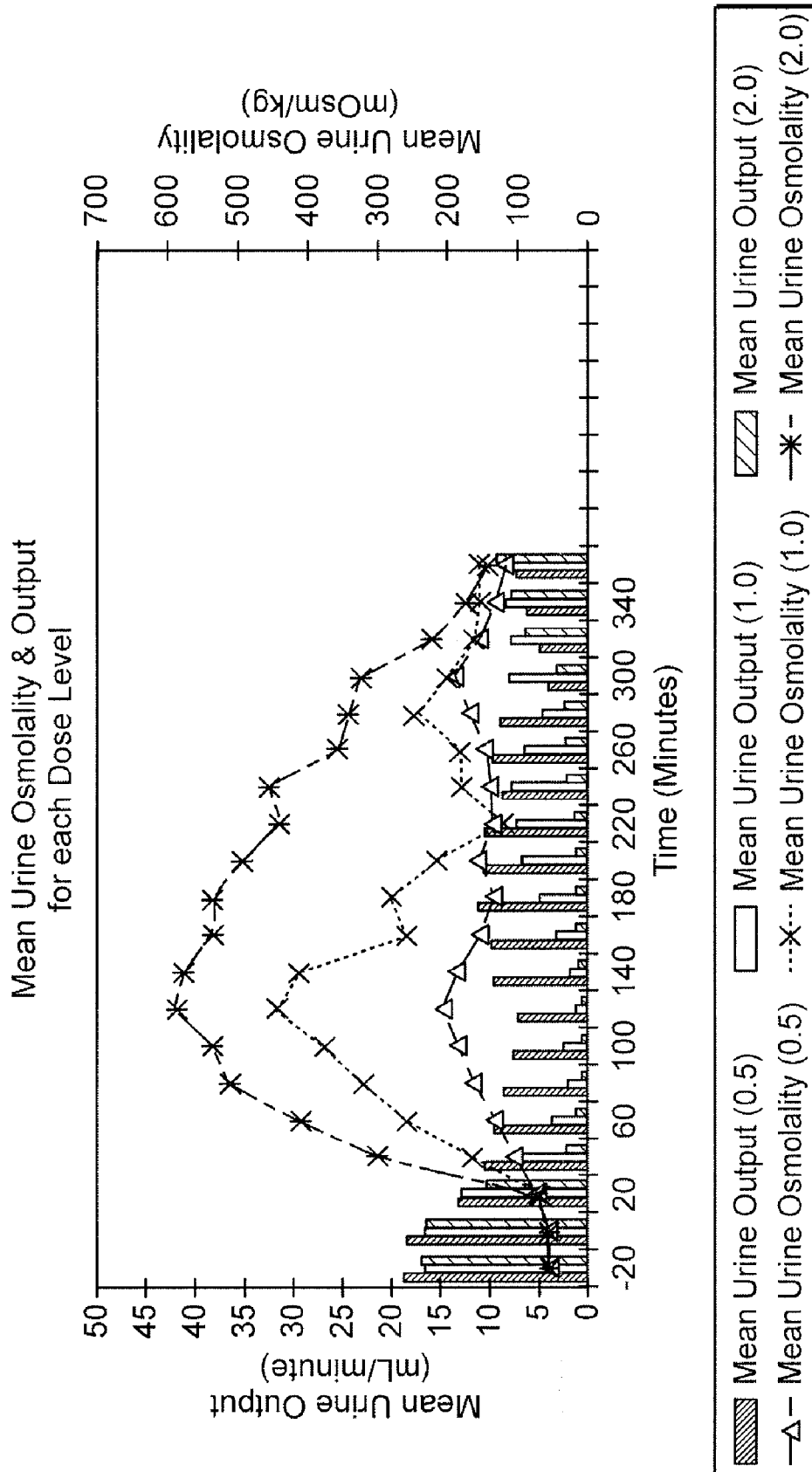


FIG. 9

1

PHARMACEUTICAL COMPOSITIONS INCLUDING LOW DOSAGES OF DESMOPRESSIN

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. patent application Ser. No. 11/744,615, filed May 4, 2007, which is a divisional application of U.S. patent application Ser. No. 10/706,100, filed Nov. 12, 2003, which is a Continuation-In-Part Application of PCT Application PCT/US03/14463, filed May 6, 2003, each of which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to pharmaceutical compositions including desmopressin, and more particularly to pharmaceutical compositions including low dosages of desmopressin for treatment of certain human diseases.

2. Brief Description of the Related Art

Desmopressin (1-desamino-8-D-arginine vasopressin, dDAVP) is an analogue of vasopressin. Desmopressin has decreased vasopressor activity and increased antidiuretic activity compared to vasopressin. This pharmacological profile enables desmopressin to be clinically used for antidiuresis without causing significant increases in blood pressure. Desmopressin is commercially available as the acetate salt both in tablet form and as a nasal spray, and is commonly prescribed for voiding postponement, incontinence, primary nocturnal enuresis (PNE) and nocturia, among other indications, including central diabetes insipidus.

Desmopressin has been administered intravenously, subcutaneously, intranasally and orally. The intravenous route of administration is clinically used almost exclusively to treat patients with mild hemophilia or Von Willebrand's Disease to raise blood levels of Factor VIII prior to surgery. Subcutaneous injection is used infrequently and primarily in patients with central diabetes insipidus, a deficiency of vasopressin resulting in the renal production of large volumes of extremely dilute urine which can cause severe dehydration. Intranasal administration of desmopressin via a nasal spray is approved for the maintenance treatment of patients with central diabetes insipidus and in children (ages 6 to 16 years) with primary nocturnal enuresis. An oral tablet dosage form of desmopressin is also approved for the treatment of central diabetes insipidus and primary nocturnal enuresis.

Currently, approved labeling for desmopressin recommends dosing in the following ranges depending on the clinical indication and the route of administration:

| Clinical Indication | Route of Administration (% Bioavailability) | Dose Range (daily) |
|----------------------------------|--|--|
| Hemophilia/Von Willebrand's | Intravenous (100) | 0.3 mcg/kg (21 mcg for 70 kg patients) |
| Central Diabetes Insipidus (CDI) | Intravenous (100) | 2-4 mcg qd or 1-2 mcg bid |
| | Subcutaneous (± 90) | 2-4 mcg qd or 1-2 mcg bid |
| | Intranasal (3-5) | 5-40 mcg qd or 5-20 mcg bid |
| | Oral (0.1) | 100-600 mcg bid |
| Primary Nocturnal Enuresis (PNE) | Intranasal (3-5) | 10-40 mcg qhs |
| | Oral (0.1) | 200-600 mcg qhs |

2

The maximum plasma/plasma/serum concentrations achieved with a typical intranasal dose of desmopressin for CDI or PNE of 20 micrograms (mcg or μg) would be approximately 20-30 pg/mL based on 3-5% bioavailability. For the desmopressin oral tablet with only 0.1-0.15% bioavailability, a standard dose of 200-400 mcg would also produce a peak plasma/plasma/serum level of 20-30 pg/mL.

While existing formulations of desmopressin have met the needs of patients, there is still a need for improvement. Tablets are often preferred by patients because of their ease of use, discretion and the lack of uncertainty of correct administration. However, tablets generally need to be taken with a glass of water or other drink, which is a problem as fluid intake needs to be restricted in connection with desmopressin treatment, and the message to the patient is much clearer when there is no water intake at all. In addition, while the above doses and plasma/plasma/serum concentrations are effective for treating CDI and PNE, standard dosages of desmopressin have been shown to cause undesirable side-effects including high incidences of hyponatremia. Lower dosages are preferable if the same desired effect could be produced. However, the current trend in this field is the evaluation of higher dosages of desmopressin for treatment purposes.

SUMMARY OF THE INVENTION

In one aspect, the present invention is directed to a pharmaceutical composition, comprising 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

In another aspect, the present invention is directed to a pharmaceutical composition, comprising desmopressin and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is effective to establish a steady plasma/plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per mL plasma/plasma/serum to about 10.00 picogram desmopressin per mL plasma/plasma/serum.

In another aspect, the present invention is directed to an article of manufacture comprising packaging material and a pharmaceutical composition contained within the packaging material, wherein the pharmaceutical composition is therapeutically effective for treating or preventing hemophilia, Von Willebrand's Disease, incontinence, primary nocturnal enuresis (PNE), nocturia, or central diabetes insipidus, and wherein the packaging material comprises a label which indicates that the pharmaceutical composition can be used for treating or preventing hemophilia, Von Willebrand's Disease, incontinence, primary nocturnal enuresis (PNE), nocturia, or central diabetes insipidus, and wherein the pharmaceutical composition comprises 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier. In another aspect, the present invention is directed to a method of treating or preventing a disease or condition which is treatable or preventable by desmopressin, the method comprising administering to a patient a daily dose of a therapeutically effective amount of a pharmaceutical composition comprising 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

In another aspect, the present invention is directed to methods of inducing antidiuretic effects in a patient, comprising the step of administering to a patient a daily dose of a therapeutically effective amount of a pharmaceutical composition comprising 0.5 ng to 20 μg desmopressin and a pharmaceutically acceptable carrier.

These and other aspects will become apparent upon reading the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

The invention will be more fully understood from the following detailed description taken in conjunction with the accompanying figures in which:

FIG. 1 shows urine osmolality for each subject as a result of administration of 0.5 ng/kg of desmopressin;

FIG. 2 shows urine osmolality for each subject as a result of administration of 1.0 ng/kg of desmopressin;

FIG. 3 shows urine osmolality for each subject as a result of administration of 2.0 ng/kg of desmopressin;

FIG. 4 shows urine output for each subject as a result of administration of 0.5 ng/kg of desmopressin;

FIG. 5 shows urine output for each subject as a result of administration of 1.0 ng/kg of desmopressin;

FIG. 6 shows urine output for each subject as a result of administration of 2.0 ng/kg of desmopressin;

FIG. 7 shows mean urine osmolality resulting from administration of 0.5, 1.0, and 2.0 ng/kg desmopressin;

FIG. 8 shows urine output resulting from administration of 0.5, 1.0, and 2.0 ng/kg desmopressin; and

FIG. 9 shows mean urine osmolality and mean urine output resulting from administration of 0.5, 1.0, and 2.0 ng/kg desmopressin.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that desmopressin can be administered as a solid dosage form which is absorbed from the mouth and which provides improved bioavailability. It is surprising that desmopressin can be absorbed at all in this way, since the available evidence showed that desmopressin administered in the oral cavity (sub-lingually) was not significantly absorbed (Fjellestad-Paulsen A. et al., *Clin. Endocrinol* 38 177-82 (1993)). It is even more unexpected that bioavailability can be improved compared to a conventional per oral tablet formulation (i.e. which is swallowed by the patient).

It has also been unexpectedly discovered that low doses and plasma/plasma/serum levels of desmopressin are pharmacologically active and can achieve desired therapeutic efficacy. The present inventor has found that doses and plasma/plasma/serum concentrations of desmopressin which are from 5 to 40% of the current recommended doses and resulting plasma/plasma/serum levels are therapeutically effective, and in some cases safer for treatment of CDI, PNE, and additional clinical indications requiring pharmacological concentration of the urine. It has been discovered that the actual dose response curve of desmopressin is shifted to the left relative to current theory and practice and that at each plasma/plasma/serum concentration point over the dose range predicted an incremental pharmacological effect in terms of urine concentration is observed.

According to a first aspect of the invention, there is provided a pharmaceutical dosage form of desmopressin adapted for sublingual absorption.

The desmopressin may be in the form of the free base or a pharmaceutically or, where appropriate veterinarily, acceptable salt, or in any other pharmaceutically or veterinarily acceptable form. The acetate salt is particularly preferred.

The formulation will typically be solid. It may disperse rapidly in the mouth. Such formulations are termed 'orodispersible'. The formulation will typically comprise a suitable carrier for this purpose, which will be pharmaceutically

acceptable (or veterinarily acceptable in the case of administration to non-human animals).

The daily dosage of desmopressin, measured as the free base, will generally be from 0.5 or 1 µg to 1 mg per dosage form. In one preferred dosage range, the dosage will typically range from 2 µg to 800 µg per dosage form and preferably from 10 µg to 600 µg. Comparatively lower doses (e.g., lower dosages relative to the dosages above or provided in the art) are also specifically contemplated, for example from 0.5 ng to 20,000 ng, preferably 0.05 mcg (50 ng) to 10 mcg (10,000 ng), and more preferably 0.1mcg (100 ng) to 2000 ng. When one dosage form per day is administered, as is usual for PNE and nocturia, this will typically be the dose per dosage form. When the daily dose is administered in two or more dosages, as will typically be the case for central diabetes insipidus, the amount of the active compound per dosage form will be reduced accordingly. The effective daily dosage will depend on the condition of the individual patient, and is thus within the ordinary skill of the art to determine for any particular patient. Other active ingredients, whether or not peptides, may also be present.

Pharmaceutical dosage forms of the present invention are adapted to supply the active ingredient to the oral cavity. The active may be absorbed across the sublingual mucosa for systemic distribution.

A variety of formulations are known which are suitable for delivering other active ingredients for absorption from the oral cavity. Such formulations may be useful in the present invention. Among them are intrabuccally disintegrating solid formulations or preparations which comprise the active ingredient, a sugar comprising lactose and/or mannitol and 0.12 to 1.2 w/w %, based on the solid components, of agar and which has a density of 400 mg/ml to 1,000 mg/ml and have a sufficient strength for handling, which in practice may mean sufficient strength to withstand removal from a blister packaging without disintegrating. Such formulations, and how to make them, are disclosed in U.S. Pat. No. 5,466,464, to which reference is made for further details.

In this embodiment of the invention, the sugar may be used in the formulation in an amount of at least 50 w/w %, preferably 80 w/w % or more, more preferably 90 w/w % or more, based on the total solid components, although it may vary depending on the quality and the quantity of the active ingredient to be used.

Though types of agar are not particularly limited, those listed in the Japanese Pharmacopoeia may be used preferably. Examples of the listed agar include agar powders PS-7 and PS-8 (manufactured by Ina Shokuhin).

Agar may be used in an amount from 0.12 to 1.2 w/w %, preferably from 0.2 to 0.4 w/w %, based on the solid components.

In order to produce a formulation in accordance with this embodiment of the present invention, a sugar comprising lactose and/or mannitol is suspended in an aqueous agar solution, filled with a mould, solidified into a jelly-like form and then dried. The aqueous agar solution may have a concentration of from 0.3 to 2.0%, preferably from 0.3 to 0.8%. The aqueous agar solution may be used in such an amount that the blending ratio of agar based on the solid components becomes 0.12 to 1.2 w/w %, but preferably 40 to 60 w/w % of agar solution based on the solid components.

Other formulations known for delivering active ingredients for absorption from the oral cavity are the dosage forms disclosed in U.S. Pat. Nos. 6,024,981 and 6,221,392. They are hard, compressed, rapidly dissolvable dosage forms adapted for direct oral dosing comprising: an active ingredient and a matrix including a non-direct compression filter and a lubri-

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cant, said dosage form being adapted to rapidly dissolve in the mouth of a patient and thereby liberate said active ingredient, and having a friability of about 2% or less when tested according to the U.S.P., said dosage form optionally having a hardness of at least about 15 Newtons (N), preferably from 15-50 N. U.S. Pat. Nos. 6,024,981 and 6,221,392 disclose further details and characteristics of these dosage forms and how to make them.

Preferably, dosage forms in accordance with this embodiment of the invention dissolve in about 90 seconds or less (preferably 60 seconds or less and most preferably 45 seconds or less) in the patient's mouth. It is also often desirable that the dosage form include at least one particle. The particle would be the active ingredient and a protective material. These particles can include rapid release particles and or sustained release particles.

In a particularly preferred formulation in accordance with this embodiment of the present invention there is provided a hard, compressed, rapidly dissolving tablet adapted for direct oral dosing. The tablet includes particles made of an active ingredient and a protective material. These particles are provided in an amount of between about 0.01 and about 75% by weight based on the weight of the tablet. The tablet also includes a matrix made from a non-direct compression filler, a wicking agent, and a hydrophobic lubricant. The tablet matrix comprises at least about 60% rapidly water soluble ingredients based on the total weight of the matrix material. The tablet has a hardness of between about 15 and about 50 Newtons, a friability of less than 2% when measured by U.S.P. and is adapted to dissolve spontaneously in the mouth of a patient in less than about 60 seconds and thereby liberate said particles and be capable of being stored in bulk.

A very fine grained or powdered sugar known as a non-direct compression sugar may be used as a filler in the matrix of this embodiment the present invention. This material, in part because of its chemical composition and in part because of its fine particle size, will dissolve readily in the mouth in a matter of seconds once it is wetted by saliva. Not only does this mean that it can contribute to the speed at which the dosage form will dissolve, it also means that while the patient is holding the dissolving dosage form in his or her mouth, the filler will not contribute a "gritty" or "sandy" texture thus adversely affecting the organoleptic sensation of taking the dosage form. In contrast, direct compression versions of the same sugar are usually granulated and treated to make them larger and better for compaction. While these sugars are water soluble, they may not be solubilised quickly enough. As a result, they can contribute to the gritty or sandy texture of the dosage form as it dissolves. Dissolution time in the mouth can be measured by observing the dissolution time of the tablet in water at about 37° C. The tablet is immersed in the water without forcible agitation or with minimal agitation. The dissolution time is the time from immersion to substantially complete dissolution of the rapidly water soluble ingredients of the tablet as determined by visual observation.

Particularly preferred fillers, in accordance with the present invention are non-direct compression sugars and sugar alcohols which meet the specifications discussed above. Such sugars and sugar alcohols include, without limitation, dextrose, mannitol, sorbitol, lactose and sucrose. Of course, dextrose, for example, can exist as either a direct compression sugar, i.e., a sugar which has been modified to increase its compressibility, or a non-direct compression sugar.

Generally, the balance of the formulation can be matrix. Thus the percentage of filler can approach 100%. However, generally, the amount of non-direct compression filler useful

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in accordance with the present invention ranges from about 25 to about 95%, preferably between about 50 and about 95% and more preferably from about 60 to about 95%.

The amount of lubricant used can generally range from between about 1 to about 2.5% by weight, and more preferably between about 1.5 to about 2% by weight. Hydrophobic lubricants useful in accordance with the present invention include alkaline stearates, stearic acid mineral and vegetable oils, glyceryl behenate and sodium stearyl fumarate. Hydrophilic lubricants can also be used.

Protective materials useful in accordance with this embodiment of the present invention may include any of the polymers conventionally utilized in the formation of microparticles, matrix-type microparticles and microcapsules. Among these are cellulosic materials such as naturally occurring cellulose and synthetic cellulose derivatives; acrylic polymers and vinyl polymers. Other simple polymers include proteinaceous materials such as gelatin, polypeptides and natural and synthetic shellacs and waxes. Protective polymers may also include ethylcellulose, methylcellulose, carboxymethyl cellulose and acrylic resin material sold under the registered trade mark EUDRAGIT by Rhone Pharma GmbH of Weierstadt, Germany.

In addition to the ingredients previously discussed, the matrix may also include wicking agents, non-effervescent disintegrants and effervescent disintegrants. Wicking agents are compositions which are capable of drawing water up into the dosage form. They help transport moisture into the interior of the dosage form. In that way the dosage form can dissolve from the inside, as well as from the outside.

Any chemical which can function to transport moisture as discussed above can be considered a wicking agent. Wicking agents include a number of traditional non-effervescent disintegration agents. These include, for example, microcrystalline cellulose (AVICEL PH 200, AVICEL PH 101), Ac-Di-Sol (Croscarmellose Sodium) and PVP-XL (a crosslinked polyvinylpyrrolidone); starches and modified starches, polymers, and gum such as Arabic and xanthan. Hydroxyalkyl cellulose such as hydroxymethylcellulose, hydroxypropylcellulose and hydroxypropylmethylcellulose, as well as compounds such as carbopol may be used as well.

The conventional range of non-effervescent disintegrant agents used in conventional tablets can be as high as 20%. However, generally, the amount of disintegration agent used ranged from between about 2 and about 5%, according to the Handbook of Pharmaceutical Excipients.

In accordance with this embodiment of the present invention, the amount of wicking agents used may range from between 2 to about 12% and preferably from between 2 to about 5%.

It is also possible, of course, to include non-effervescent disintegrants which may not act to wick moisture, if desirable. In either event, it is preferable to use either rapidly water soluble, non-effervescent disintegrants or wicking agents and/or to minimize the use of generally non-water soluble wicking agents or non-effervescent disintegrants. Non-rapidly dissolvable, non-rapidly water soluble elements if used in sufficient quantity, can adversely affect the organoleptic properties of the tablets as they dissolve within the mouth and therefore should be minimized. Of course, wicking agents or non-effervescent disintegrants which are rapidly water soluble as discussed herein can be used in greater quantity and they will not add to the grittiness of the formulation during dissolution. Preferred wicking agents in accordance with the present invention include crosslinked PVP, although, the amounts of these must be controlled as they are not rapidly water soluble.

In addition, it may be desirable to use an effervescent couple, in combination with the other recited ingredients to improve the disintegration profile, the organoleptic properties of the material and the like. Preferably, the effervescent couple is provided in an amount of between about 0.5 and about 50%, and more preferably, between about 3 and about 15% by weight, based on the weight of the finished tablet. It is particularly preferred that sufficient effervescent material be provided such that the evolved gas is less than about 30 cm, upon exposure to an aqueous environment.

The term "effervescent couple" includes compounds which evolve gas. The preferred effervescent couple evolve gas by means of a chemical reaction which takes place upon exposure of the effervescent disintegration couple to water and/or to saliva in the mouth. This reaction is most often the result of the reaction of a soluble acid source and an alkali monohydrogencarbonate or other carbonate source. The reaction of these two general compounds produces carbon dioxide gas upon contact with water or saliva. Such water-activated materials must be kept in a generally anhydrous state and with little or no absorbed moisture or in a stable hydrated form, since exposure to water will prematurely disintegrate the tablet. The acid sources may be any which are safe for human consumption and may generally include food acids, acid and hydrite antacids such as, for example: citric, tartaric, malic, fumaric, adipic, and succinics. Carbonate sources include dry solid carbonate and bicarbonate salt such as, preferably, sodium bicarbonate, sodium carbonate, potassium bicarbonate and potassium carbonate, magnesium carbonate and the like. Reactants which evolve oxygen or other gasses and which are safe for human consumption are also included.

In the case of the orally dissolvable tablets in accordance with the present invention, it is preferred that both the amount and the type of disintegration agent, either effervescent or non-effervescent, and the combination thereof be provided sufficient in a controlled amount such that the tablet provides a pleasant organoleptic sensation in the mouth of the patient. In some instances, the patient should be able to perceive a distinct sensation of fizzing or bubbling as the tablet disintegrates in the mouth. In general, the total amount of wicking agents, non-effervescent disintegrants and effervescent disintegrants should range from 0-50%. However, it should be emphasized that the formulations of the present invention will dissolve rapidly and therefore, the need for disintegrating agents is minimal. As illustrated in the examples, appropriate hardness, friability and dissolution times can be obtained even without effervescent disintegrants or high quantities of wicking agents.

The use of a non-direct compression filler eliminates the need for many conventional processing steps such as granulation and/or the need to purchase more expensive pre-granulated, compressible fillers. At the same time, the resulting dosage form is a balance of performance and stability. It is robust enough to be conventionally produced using direct compression. It is robust enough to be stored or packaged in bulk. Yet, it rapidly dissolves in the mouth while minimizing the unpleasant feel of conventional disintegrating tablets to the extent possible.

Formulations in accordance with the embodiment of the invention may be made by a method including the steps of:

- (a) forming a mixture including an active ingredient and a matrix including a non-direct compression filler and a lubricant;

- (b) compressing the mixture to form a plurality of hard, compressed, rapidly disintegrable dosage forms including the active ingredient distributed in the orally dissolvable matrix; and optionally

- (c) storing the dosage forms in bulk prior to packaging. In a preferred embodiment, the dosage forms are then packaged in a lumen of a package such that there is at least one per package. In a preferred particularly preferred embodiment, the dosage forms are then packaged in a lumen of a package such that there more than one per package. Direct compression is the preferred method of forming the dosage forms.

Other formulations known for delivering active ingredients for absorption from the oral cavity are the dosage forms disclosed in U.S. Pat. No. 6,200,604, which comprise an orally administrable medicament in combination with an effervescent agent used as penetration enhancer to influence the permeability of the medicament across the buccal, sublingual, and gingival mucosa. In the content of the present invention, the medicament is desmopressin, which is administered in most embodiments across the sublingual mucosa. In the formulations of this embodiment of the invention, effervescent agents can be used alone or in combination with other penetration enhancers, which leads to an increase in the rate and extent of oral absorption of an active drug.

Formulations or dosage forms in accordance with this embodiment of the invention should include an amount of an effervescent agent effective to aid in penetration of the drug across the oral mucosa. Preferably, the effervescent is provided in an amount of between about 5% and about 95% by weight, based on the weight on the finished tablet, and more preferably in an amount of between about 30% and about 80% by weight. It is particularly preferred that sufficient effervescent material be provided such that the evolved gas is more than about 5 cm³ but less than about 30 cm³, upon exposure of the tablet to an aqueous environment.

The term "effervescent agent" includes compounds which evolve gas. The preferred effervescent agents evolve gas by means of a chemical reaction which takes place upon exposure of the effervescent agent (an effervescent couple) to water and/or to saliva in the mouth. This reaction is most often the result of the reaction of a soluble acid source and a source of carbon dioxide such as an alkaline carbonate or bicarbonate. The reaction of these two general compounds produces carbon dioxide gas upon contact with water or saliva. Such water-activated materials must be kept in a generally anhydrous state and with little or no absorbed moisture or in a stable hydrated form, since exposure to water will prematurely disintegrate the tablet. The acid sources may be any which are safe for human consumption and may generally include food acids, acid and hydrite antacids such as, for example: citric, tartaric, malic, fumaric, adipic, and succinics. Carbonate sources include dry solid carbonate and bicarbonate salt such as, preferably, sodium bicarbonate, sodium carbonate, potassium bicarbonate and potassium carbonate, magnesium carbonate and the like. Reactants which evolve oxygen or other gasses and which are safe for human consumption are also included.

The effervescent agent(s) useful in this embodiment of the present invention is not always based upon a reaction which forms carbon dioxide. Reactants which evolve oxygen or other gasses which are safe for human consumption are also considered within the scope. Where the effervescent agent includes two mutually reactive components, such as an acid source and a carbonate source, it is preferred that both components react completely. Therefore, an equivalent ratio of components which provides for equal equivalents is pre-

ferred. For example, if the acid used is diprotic, then either twice the amount of a mono-reactive carbonate base, or an equal amount of a di-reactive base should be used for complete neutralization to be realized. However, in other embodiments of the present invention, the amount of either acid or carbonate source may exceed the amount of the other component. This may be useful to enhance taste and/or performance of a tablet containing an overage of either component. In this case, it is acceptable that the additional amount of either component may remain unreacted.

Such dosage forms may also include the amounts additional to that required for effervescence a pH adjusting substance. For drugs that are weakly acidic or weakly basic, the pH of the aqueous environment can influence the relative concentrations of the ionized and unionized forms of the drug present in solution according to the Henderson-Hasselbach equation. The pH solutions in which an effervescent couple has dissolved is slightly acidic due to the evolution of carbon dioxide. The pH of the local environment, e.g. saliva in immediate contact with the tablet and any drug that may have dissolved from it, may be adjusted by incorporating in the tablet a pH adjusting substances which permit the relative portions of the ionized and unionized forms of the drug to be controlled. In this way, the present dosage forms can be optimized for each specific drug. If the unionized drug is known or suspected to be absorbed through the cell membrane (transcellular absorption) it would be preferable to alter the pH of the local environment (within the limits tolerable to the subject) to a level that favours the unionized form of the drug. Conversely, if the ionized form is more readily dissolved the local environment should favour ionization.

The aqueous solubility of the drug should preferably not be compromised by the effervescent and pH adjusting substance, such that the dosage forms permit a sufficient concentration of the drug to be present in the unionized form. The percentage of the pH adjusting substance and/or effervescent should therefore be adjusted depending on the drug.

Suitable pH adjusting substance for use in the present invention include any weak acid or weak base in amounts additional to that required for the effervescence or, preferably, any buffer system that is not harmful to the oral mucosa. Suitable pH adjusting substance for use in the present invention include, but are not limited to, any of the acids or bases previously mentioned as effervescent compounds, disodium hydrogen phosphate, sodium dihydrogen phosphate and the equivalent potassium salt.

The dosage form of this embodiment of the invention preferably includes one or more other ingredients to enhance the absorption of the pharmaceutical ingredient across the oral mucosa and to improve the disintegration profile and the organoleptic properties of the dosage form. For example, the area of contact between the dosage form and the oral mucosa, and the residence time of the dosage form in the oral cavity can be improved by including a bioadhesive polymer in this drug delivery system. See, for example, Mechanistic Studies on Effervescent-Induced Permeability Enhancement by Jonathan Eichman (1997), which is incorporated by reference herein. Effervescence, due to its mucus stripping properties, would also enhance the residence time of the bioadhesive, thereby increasing the residence time for the drug absorption. Non-limiting examples of bioadhesives used in the present invention include, for example, Carbopol 934 P, Na CMC, Methocel, Polycarbophil (Noveon AA-1), HPMC, Na alginate, Na Hyaluronate and other natural or synthetic bioadhesives.

In addition to the effervescence-producing agents, a dosage form according to this embodiment of the present inven-

tion may also include suitable non-effervescent disintegration agents. Non-limiting examples of non-effervescent disintegration agents include: microcrystalline, cellulose, croscarmellose sodium, crospovidone, starches, corn starch, potato starch and modified starches thereof, sweeteners, clays, such as bentonite, alginates, gums such as agar, guar, locust bean, karaya, pectin and tragacanth. Disintegrants may comprise up to about 20 weight percent and preferably between about 2 and about 10% of the total weight of the composition.

In addition to the particles in accordance with this embodiment of the present invention, the dosage forms may also include glidants, lubricants, binders, sweeteners, flavouring and colouring components. Any conventional sweetener or flavouring component may be used. Combinations of sweeteners, flavouring components, or sweeteners and flavouring components may likewise be used.

Examples of binders which can be used include acacia, tragacanth, gelatin, starch, cellulose materials such as methyl cellulose and sodium carboxy methyl cellulose, alginic acids and salts thereof, magnesium, aluminium silicate, polyethylene glycol, guar gum, polysaccharide acids, bentonites, sugars, invert sugars and the like. Binders may be used in an amount of up to 60 weight percent and preferably about 10 to about 40 weight percent of the total composition.

Colouring agents may include titanium dioxide, and dyes suitable for food such as those known as F.D. & C. dyes and natural coloring agents such as grape skin extract, beet red powder, beta-carotene, annatto, carmine, turmeric, paprika, etc. The amount of colouring used may range from about 0.1 percent to about 3.5 weight percent of the total composition.

Flavours incorporated in the composition may be chosen from synthetic flavours oils and flavouring aromatics and/or natural oils, extracts from plants, leaves, flowers, fruits and so forth and combinations thereof. These may include cinnamon oil, oil of wintergreen, peppermint oils, clove oil, bay oil anise oil, eucalyptus, thyme oil, cedar leave oil, oil of nutmeg, oil of sage, oil of bitter almonds and cassia oil. Also useful as flavours are vanilla, citrus oil, including lemon, orange, grape, lime and grapefruit, and fruit essences, including apple, pear, peach, strawberry, raspberry, cherry, plum, pineapple, apricot and so forth. Flavours which have been found to be particularly useful include commercially available orange, grape, cherry and bubble gum flavours and mixtures thereof. The amount of flavouring may depend on a number of factors, including the organoleptic effect desired. Flavours may be present in an amount ranging from about 0.05 to about 3 percent by weight based upon the weight of the composition. Particularly preferred flavours are the grape and cherry flavours and the citrus flavours such as orange.

One aspect of the invention provides a solid, oral tablet dosage form suitable for sublingual administration. Excipient fillers can be used to facilitate tableting. The filler desirably will also assist in the rapid dissolution of the dosage form in the mouth. Non-limiting examples of suitable fillers include: mannitol, dextrose, lactose, sucrose, and calcium carbonate.

As described in U.S. Pat. No. 6,200,604, tablets can either be manufactured by direct compression, wet granulation or any other tablet manufacturing technique. The dosage form may be administered to a human or other mammalian subject by placing the dosage form in the subject's mouth and holding it in the mouth, beneath the tongue (for sublingual administration). The dosage form spontaneously begins to disintegrate due to the moisture in the mouth. The disintegration, particularly the effervescence, stimulates additional salivation which further enhances disintegration.

Although the above described formulations are within the scope of the present invention, the most preferred orodispersible solid pharmaceutical dosage forms according to the invention comprise a pharmaceutically active peptide and an open matrix network carrying desmopressin, the open matrix network being comprised of a water-soluble or water-dispersible carrier material that is inert towards desmopressin.

Pharmaceutical dosage forms comprising open matrix networks are known from GB-A-1548022, to which reference is made for further details. Pharmaceutical dosage forms of the invention can be rapidly disintegrated by water. By "rapidly disintegrated" is meant that the shaped articles are disintegrated in water within 10 seconds. Preferably the shaped article disintegrates (dissolves or disperses) within 5 seconds or less. The disintegration time is measured by a procedure analogous to the Disintegration Test for Tablets, B.P. 1973. The procedure is described in GB-A-1548022 and outlined below.

Apparatus

A glass or suitable plastic tube 80 to 100 mm long, with an internal diameter of about 28 mm and an external diameter of 30 to 31 mm, and fitted at the lower end, so as to form a basket, with a disc of rustproof wire gauze complying with the requirements for a No. 1.70 sieve.

A glass cylinder with a flat base and an internal diameter of about 45 mm containing water not less than 15 cm deep at a temperature between 36° and 38° C.

The basket is suspended centrally in the cylinder in such a way that it can be raised and lowered repeatedly in a uniform manner so that at the highest position the gauze just breaks the surface of the water and at the lowest position the upper rim of the basket just remains clear of the water.

Method

Place one shaped article in the basket and raise and lower it in such a manner that the complete up and down movement is repeated at a rate equivalent to thirty times a minute. The shaped articles are disintegrated when no particle remains above the gauze which would not readily pass through it. No such particle should remain after 10 seconds.

By the term "open matrix network" there is meant a network of water-soluble or water-dispersible carrier material having interstices dispersed throughout. The open matrix network of carrier material is of generally low density. For example the density may be within the range 10 to 200 mg/cc e.g. 10 to 100 mg/cc, preferably 30 to 60 mg/cc. The density of the shaped article may be affected by the amount of active ingredient, or any other ingredients, incorporated into the article and may be outside the above mentioned preferred limits for the density of the matrix network. The open matrix network which is similar in structure to a solid foam enables a liquid to enter the product through the interstices and permeate through the interior. Permeation by aqueous media exposes the carrier material of both the interior and exterior of the product to the action of the aqueous media whereby the network of carrier material is rapidly disintegrated. The open matrix structure is of a porous nature and enhances disintegration of the product as compared with ordinary solid shaped pharmaceutical dosage forms such as tablets, pills, capsules, suppositories and pessaries. Rapid disintegration results in rapid release of the active ingredient carried by the matrix.

The carrier material used in the product of the invention may be any water-soluble or water-dispersible material that is pharmacologically acceptable or inert to the chemical and which is capable of forming a rapidly disintegratable open matrix network. It is preferred to use water-soluble material as the carrier since this results in the most rapid disintegration

of the matrix when the product is placed in an aqueous medium. A particularly advantageous carrier may be formed from polypeptides such as gelatin, particularly gelatin which is particularly hydrolysed, e.g. by heating in water. For example, the gelatin may be partially hydrolysed by heating a solution of the gelatin in water, e.g. in an autoclave at about 120° C. for up to 2 hours, e.g. from about 5 minutes to about 1 hour, preferable from about 30 minutes to about 1 hour. The hydrolysed gelatin is preferably used at concentrations of about 1 to 6% weight/vol., most preferably at 2 to 4% e.g. about 3%.

Although mammalian derived gelatin may be used, it has an unpleasant taste and thus necessitates the use of sweeteners and flavours to mask the taste of the gelatin in addition to any sweeteners and flavours which may be required to mask the taste of the active ingredient. Moreover, the heating step necessary with the use of mammalian gelatin increases processing times and incurs heating costs thereby increasing the overall costs of the process. Therefore, the use of fish gelatin, especially non-gelling fish gelatin, is preferred, especially for desmopressin. Reference is made to WO-A-0061117 for further details.

Other carrier materials may be used in place of partially hydrolysed gelatin or fish gelatin, for example polysaccharides such as hydrolysed dextran, dextrin and alginates (e.g. sodium alginate) or mixtures of above mentioned carriers with each other or with other carrier materials such as polyvinyl alcohol, polyvinylpyrrolidone or acacia. Modified starch may also be used in place of gelatin, as described in WO-A-0044351, to which reference is made for further details. Additional carriers include water, lactose, starch, magnesium stearate, talc, plant oils, gums, alcohol, Vaseline (petroleum jelly), or the like.

Pharmaceutical dosage forms of the invention may be in the form of shaped articles. They may incorporate ingredients in addition to the active ingredient(s). For example the pharmaceutical dosage form of the present invention may incorporate pharmaceutically acceptable adjuvants. Such adjuvants include, for example, colouring agents, flavouring agents, preservatives (e.g. bacteriostatic agents), and the like. U.S. Pat. No. 5,188,825 teaches that water soluble active agents should be bonded to an ion exchange resin to form a substantially water insoluble active agent/resin complex; although that teaching may be practiced here (for which reference to U.S. Pat. No. 5,188,825 is made for further details), it has been found in the development of the present invention that water soluble peptides such as desmopressin may be formulated in solid dosage forms of the invention without the need for bonding to an ion exchange resin. Such dosage forms may therefore be free of an ion exchange resin. For hydrophobic peptides, which desmopressin is not, a surfactant may be present, as taught in U.S. Pat. No. 5,827,541, to which reference is made for further details. For peptides with an unpleasant taste (which desmopressin does not have), a lipid such as a lecithin may be present to improve patient acceptability, as taught in U.S. Pat. No. 6,156,339, to which reference is made for further details. Other strategies for taste masking include conversion of a soluble salt to a less soluble salt or to the free base, as taught by U.S. Pat. Nos. 5,738,875 and 5,837,287, and the use of a process disclosed in U.S. Pat. No. 5,976,577 wherein, prior to freeze drying, a suspension of uncoated or coated coarse particles of the pharmaceutically active substance(s) in a carrier material is cooled to reduce the viscosity and minimize release of the active substance during processing, as well as beyond the point of disintegration of the form in the mouth, to minimize bad taste from the peptide; reference is made to the cited patents for further details.

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For insoluble or poorly soluble peptides having a large particle size, xanthan gum may be present, particularly when the carrier is formed from gelatin, as the xanthan gum may act as a gelatin flocculating agent, as disclosed in U.S. Pat. No. 5,631,023, to which reference is made for further details.

As taught by WO-A-9323017 one or more amino acids having from about 2 to 12 carbon atoms may be present, when the matrix is selected from the group consisting of gelatin, pectin, soy fibre protein and mixtures thereof. In this formulation the preferred amino acid is glycine, while the preferred matrix forming agent is gelatin and/or pectin; in a particularly preferred embodiment, the dosage form additionally comprises mannitol. All excipients will be chosen to be pharmaceutically acceptable.

Pharmaceutical dosage forms of the present invention may be prepared by a process as described in GB-A-1548022, which comprises subliming solvent from a composition comprising the pharmaceutical substance and a solution of the carrier material in a solvent, the composition being in the solid state in a mould.

The sublimation is preferably carried out by freeze drying a composition comprising the active ingredient and a solution of the carrier material in a solvent. The composition may include additional ingredients, such as those mentioned above. The solvent is preferably water but it may contain a co-solvent (such as an alcohol e.g. tert-butyl alcohol) to improve the solubility of the chemical. The composition may also contain a surfactant e.g. Tween 80 (polyoxyethylene (20) sorbitan mono-oleate). The surfactant may help to prevent the freeze dried product sticking to the surface of the mould. It may also aid in the dispersion of the active ingredient.

The composition may contain a pH adjusting agent to adjust the pH of a solution from which the dosage form is prepared within the range of from 3 to 6, preferably from 3.5 to 5.5, and most preferably from 4 to 5, for example 4.5 or 4.8. Citric acid is a preferred pH adjusting agent, but others including hydrochloric acid, malic acid can be used. Such non-volatile pH adjusting agents will not be removed by the freeze drying or other sublimation process and so may be present in the final product.

The mould may comprise a series of cylindrical or other shape depressions in it, each of a size corresponding to the desired size of the shaped article. Alternatively, the size of the depression in the mould may be larger than the desired size of the article and after the contents have been freeze dried the product can be cut into the desired size (for example thin wafers).

However, as described in GB-A-2111423, the mould is preferably a depression in a sheet of filmic material. The filmic material may contain more than one depression. The filmic material may be similar to that employed in conventional blister packs which are used for packaging oral contraceptive tablets and like medicament forms. For example the filmic material may be made of thermoplastic material with the depressions formed by thermoforming. The preferred filmic material is a polyvinyl chloride film. Laminates of filmic material may also be used.

In one embodiment the mould comprises a metal plate (e.g. an aluminium plate) containing one or more depressions. In a preferred process using such a mould, the mould is cooled with a cooling medium (e.g. liquid nitrogen or solid carbon dioxide). When the mould is cooled a predetermined amount of water containing the carrier material, the active ingredient and any other desired ingredient is fed into the depression(s). When the contents of the depression(s) are frozen the mould is subjected to reduced pressure and, if desired, controlled application of heat to aid the sublimation. The pressure can be

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below about 4 mm. Hg; GB-A-1548022 teaches the employment of pressures of below 0.3 mm Hg, for example 0.1 to 0.2 mm is preferred. The freeze dried products may be removed from the depressions in the mould and stored for future use, e.g. in airtight jars or other suitable storage containers. Alternatively, the freeze dried product may be enclosed by filmic material as described in GB-A-2111423.

A later developed process useful for making pharmaceutical dosage forms in accordance with the invention is described in GB-A-2111423, to which reference is made for further details. The process comprises filling a composition comprising a predetermined amount of active ingredient and a solution of partially hydrolysed gelatin into a mould, freezing the composition in the mould by passing gaseous cooling medium over the mould and then subliming solvent from the frozen composition so as to produce a network of partially hydrolysed gelatin carrying the active ingredient.

In order to help ensure an even thickness of product, the side wall or walls of the mould may diverge outwards from the base and making an angle with the vertical of at least 5° at the surface of the composition, as described in GB-A-2119246 to which reference is made for further details.

Alternatively or in addition, pharmaceutical dosage forms of the present invention may be prepared by a process as described in GB-A-2114440 which comprises freezing a composition comprising a solution in a first solvent of a water-soluble or water dispersible carrier material that is inert towards the active ingredient, subliming the first solvent from the frozen composition so as to produce a product having a network of carrier material, adding to said product a solution or suspension of a second non-aqueous solvent containing a predetermined amount of the active ingredient and allowing or causing the second solvent to evaporate. Reference is made to GB-A-2114440 for further details.

Alternatively or in addition, pharmaceutical dosage forms of the present invention may be prepared by a process as described in GB-A-2111184, which comprises introducing the liquid medium in the form of droplets beneath the surface of a cooling liquid which is maintained at a temperature lower than the freezing point of the liquid medium, the cooling liquid being immiscible with, and inert with respect to, the liquid medium and having a density greater than that of both the liquid medium and the resulting frozen particles such as the liquid droplets float upwards in the cooling liquid towards the surface thereof, they are frozen to form spherical particles. The frozen spherical particles can be collected at or near the upper surface of the cooling liquid. Reference is made to GB-A-2111184 for further details.

Dosage forms in accordance with the invention have improved bioavailability. They are intended to be taken orally, and are highly suitable for that purpose. They disperse rapidly in the mouth, and may for example be placed under the tongue (sub-lingually).

According to a second aspect of the invention, there is provided a dosage form as described above for use in medicine, particularly, for voiding postponement, incontinence, primary nocturnal enuresis (PNE), nocturia and central diabetes insipidus.

The invention provides a method of postponing voiding, treating or preventing incontinence, primary nocturnal enuresis (PNE), nocturia and/or central diabetes insipidus, the method comprising administering an effective and generally non-toxic amount of desmopressin to a subject across the sublingual mucosa, for example in a dosage form as described above. Any other disease or condition treatable or preventable by desmopressin may similarly be addressed by means of invention. The invention therefore extends to the use of des-

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mopressin in the manufacture of a sublingually absorbable pharmaceutical formulation. The invention also extends to a pack comprising a sublingually absorbable pharmaceutical dosage form of desmopressin together with instructions to place the dosage form under a patient's tongue.

Encompassed within the invention is also a method for preparing a packaged dosage form of desmopressin, the method comprising bringing into association a sublingually absorbable pharmaceutical dosage form of desmopressin and instructions to place the dosage form under a patient's tongue. The instructions may for example be printed on packaging encompassing the dosage form when sold or dispensed, or may be on a product information leaflet or insert within the packaging.

Other peptides apart from desmopressin are formulatable in the formulations described above. The invention therefore extends to a pharmaceutical dosage form of a pharmaceutically active peptide adapted for oral absorption.

According to a further aspect of the invention, there is provided a solid pharmaceutical dosage form, for example for oral administration, the dosage form comprising a pharmaceutically active peptide and an open matrix network carrying the peptide, the open matrix network being comprised of a water-soluble or water-dispersible carrier material that is inert towards the peptide.

Although oral vaccines made from fast dissolving dosage forms are known from WO-A-9921579, there is no disclosure of pharmaceutically active peptides retaining their activity after administration. The experimental work in WO-A-9921579 merely shows the presence in saliva of IgA antibodies to tetanus toxoid following the administration of tetanus toxoid by means of an adjuvanted fast dissolving dosage vaccine formulation. Formulations of the present invention are not vaccines and do not include adjuvants.

Pharmaceutical dosage forms of this aspect of the invention contain a pharmaceutically active peptide. Such peptides may be directly active per se or they may have one or more active metabolites, i.e. they may be prodrugs for the primary or true active principle. The peptides may have for example from 2 to 20, preferably from 5 to 15, amino acid residues (at least some of which may be D-isomer, although L-isomers will generally be predominant). The peptides may be linear, branched or cyclic, and may include natural residues or substituents or residues or substituents not found in natural peptides or proteins either commonly or at all. Pharmaceutically acceptable salts, simple adducts and tautomers are included where appropriate.

Examples of peptides usefully formulated by means of the invention include somatostatin and its analogues including Cyclo(MeAla-Tyr-D-Trp-Lys-Val-Phe) and Cyclo(Asn-Phe-Phe-D-Trp-D-Lys-Thr-Phe-GABA), enkephalins including Met⁵-enkephalin and Leu⁵-enkephalin, oxytocin analogues such as atosiban (1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Om-oxytocin), GnRH analogues such as triptorelin (6-D-Trp-GnRH), leuprolide ([D-Leu⁶, Pro⁸-NHET]-GnRH), degarelix (Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(L-Hydroxyrotyl)-D-4Aph(Cbm)-Leu-Ilys-Pro-D-Ala-NH₂, where 2Nal is 2-naphthylalanine, 4Cpa is 4-chlorophenylalanine, 3Pal is 3-pyridylalanine, Ilys is N(8)-isopropyllysine, 4Aph is 4-aminophenylalanine and Cbm is the carbamoyl group) and other GnRH antagonists disclosed in U.S. Pat. Nos. 5,925, 730 and 4,072,668, and vasopressin analogues such as desmopressin. It is particularly preferred to formulate by means of the invention agonists of naturally active peptides, such as those described above, since agonists may be active at lower doses than antagonists

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Dosage will be as determined by the physician or clinician, depending on the nature of the peptide, the nature of the disease or condition being treated or prevented, and other factors.

The invention extends to the use of a peptide in the manufacture of a dosage form as described above for treating or preventing a disease or condition which is treatable or preventable by a peptide.

The invention also provides a method of preventing a disease or condition which is treatable or preventable by a peptide, the method comprising administering an effective and generally non-toxic amount of the peptide to a subject in a dosage form as described above.

Low Dosage Analysis and Applications

As indicated above, doses and plasma/plasma/serum concentrations of desmopressin which are from 5 to 40% of the current recommended doses and resulting plasma/plasma/serum levels are therapeutically effective and in some cases safer for certain disease conditions such as CDI, PNE, and additional clinical indications requiring pharmacological concentration of the urine.

Clinical observations in adult males and females treated with desmopressin for a condition known as nocturia (which results in frequent night time urination) suggested that lower dosages of desmopressin would be desirable. In this patient population, standard intranasal and oral doses of desmopressin produced an unexpectedly high incidence of hyponatremia, a condition in which plasma/plasma/serum sodium falls to abnormally low levels. Hyponatremia can result in seizures, cardiac arrhythmias, cerebral edema and death. The oral doses of desmopressin were in the 100 to 400 mcg range and the intranasal doses were in the 10 to 20 mcg range. While these doses decreased the incidence of nocturia, the hyponatremia suggested that the doses were unnecessarily high resulting in an excessive duration of pharmacodynamic effect on urine concentration with consequent overhydration and dilutional lowering of plasma/plasma/serum sodium. Lower doses of desmopressin would produce adequate but not excessive antidiuresis in terms of the magnitude and duration of action.

In accordance with the present invention, plasma/plasma/serum desmopressin concentrations following administration of the pharmaceutical composition of the invention preferably range from about 0.1 pg/mL to about 10.0 pg/mL, and more preferably from about 0.5 pg/mL to about 5.0 pg/mL. These amounts and ranges of desmopressin may be administered by any method known in the art, including, without limitation, intravenous (bolus, infusion); subcutaneous (bolus, infusion, depot); intranasal; transmucosal (buccal and sublingual, e.g., orodispersible tablets, wafers, film, and effervescent formulations; conjunctival (eyedrops); rectal (suppository, enema)); transdermal (passive via patch, gel, cream, ointment or iontophoretic); or intradermal (bolus, infusion, depot) as outlined below. Additionally, pharmaceutical compositions that contain desmopressin in an amount that provide the above plasma/plasma/serum desmopressin levels may be prepared by the above methods and using the above carriers, or any other method known in the art.

The dose ranges of desmopressin outlined above can produce appropriate antidiuretic effect when administered by various routes as summarized in the examples below:

| Route of Administration | Effective Daily Dose Range |
|---|----------------------------|
| Intravenous (bolus and infusion) | 0.5 ng-2000 ng |
| Subcutaneous (bolus, infusion, depot) | 0.5 ng-2000 ng |
| Intranasal | 0.1 mcg-20 mcg |
| Transmucosal including buccal and sublingual (orodispersible tablets, wafers, film and effervescent formulations), conjunctival (eyedrops), rectal (suppository, enema) | 0.1 mcg-20 mcg |
| Transdermal (passive via patch, gel, cream, ointment or iontophoretic) | 0.05 mcg-10 mcg |
| Intradermal (bolus, infusion, depot) | 0.05 mcg-10 mcg |

Administration of low dosages of desmopressin can be an effective treatment regimen for clinical indications such as treatment of central diabetes insipidus, prevention of primary nocturnal enuresis, prevention of nocturia, treatment of clinical disorders associated with nocturia including but not limited to sleep disturbances, prevention of incontinence (stress, urge, and the like), and voiding postponement during waking hours.

Specific formulations of desmopressin may also be created which enhance absorption and increase its systemic bioavailability. These formulations can result in incremental pharmacological effects at each point along the dose response curve, thus amplifying the activity of even low doses of desmopressin.

EXAMPLES

The present invention is further described in detail by means of the following Examples. All parts and percentages are by weight unless explicitly stated otherwise.

Example 1

200 µg Desmopressin Orodispersible Dosage Form

Spray-dried fish gelatin (4 g) and mannitol (3 g) are added to a glass beaker. Purified water (93 g) is then added and solution effected by stirring using a magnetic follower. The pH is checked and adjusted to 4.8 with citric acid as necessary. A Gilson pipette can then be used to deliver 500 mg of this solution into each one of a series of pre-formed blister pockets having a pocket diameter of about 16 mm. The blister laminate may comprise PVC coated with PVdC. The dosed units are then frozen at a temperature of -110° C. in a freeze tunnel with a residence time of 3.2 minutes and the frozen units are then held in an upright freezer for a time greater than 1.5 hours at a temperature of -25° C. (±5° C.). The units are then freeze-dried overnight with an initial shelf temperature of 10° C. rising to +20° C. at a pressure of 0.5 mbar. The units can be checked for moisture prior to unloading by the drying trace and by the pressurized moisture check.

In this way, following the general procedure given in Example 1 of WO-A-0061117, a desmopressin orodispersible dosage form is prepared using the following ingredients per unit dosage form:

| | |
|---|--------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 200 µg |
| Mannitol EP/USP (Roquette, Mannitol 35) | 15 mg |
| Fish gelatin USNF/EP | 20 mg |

-continued

| | |
|---|-----------------------------|
| Citric acid (if necessary) (pH adjusting agent) | q.s. to pH 4.8 |
| Purified water | (Removed during processing) |

Example 2

400 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 1 herein is followed, except that the amount of desmopressin per unit dosage form was 400 µg.

Example 3

800 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 1 herein is followed, except that the amount of desmopressin per unit dosage form was 800 µg.

Example 4

200 µg Desmopressin Orodispersible Dosage Form

Following the general procedure given in Example 1 of WO-A-0061117, a desmopressin dosage form orodispersible dosage form was prepared using the following ingredients per unit dosage form:

| | |
|---|-----------------------------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 200 µg |
| Mannitol EP/USP (Roquette, Mannitol 35) | 6 mg |
| Fish gelatin USNF/EP | 10 mg |
| Citric acid (if necessary) (pH adjusting agent) | q.s. to pH 4.8 |
| Purified water | (Removed during processing) |

Example 5

400 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 4 herein was followed, except that the amount of desmopressin per unit dosage form was 400 µg.

Example 6

800 µg Desmopressin Orodispersible Dosage Form

The procedure of Example 4 herein was followed, except that the amount of desmopressin per unit dosage form was 800 µg.

Comparative Example 1

Desmopressin i.v. Solution

An injectable preparation of desmopressin was conventionally prepared using the following ingredients:

| | |
|---|------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 4 mg |
| Sodium chloride | 9 mg |

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-continued

| | |
|--|--------------|
| (National Corporation of Swedish Pharmacies, Sweden) | |
| Hydrochloric acid (1N) (Merck, Germany) | q.s. to pH 4 |
| Water for injection | q.s. to 1 ml |

Comparative Example 2

200 µg Desmopressin Conventional Tablet

Using a conventional wet granulation process, tablets containing the following ingredients were prepared:

| | |
|---|-----------------------------|
| Desmopressin (PolyPeptide Laboratories, Sweden) | 200 µg |
| Lactose (Pharmatose 150M, DMV, The Netherlands) | 120 mg |
| Potato starch (Lyckeby AB, Sweden) | 77 mg |
| PVP (Kollidon 25, BASF, Germany) | 1.8 mg |
| Magnesium stearate (Peter Greven, Germany) | 1 mg |
| Granulation Liquid (water, ethanol) | (Removed during processing) |

Comparative Example 3

100 µg Desmopressin Conventional Tablet

The procedure of Comparative Example 2 was followed, except that the amount of desmopressin was 100 µg per tablet.

Example 7

Bioavailability Of Desmopressin Administered in Accordance with Examples 4 to 6

Study Design

Twenty-four healthy non-smoking male volunteers were enrolled in the present study. The study was designed as a one-centre, open-labelled, randomized, balanced, 4-way cross-over phase I study. Each subject was, in a randomized order, administered sublingually desmopressin as a 200 µg, 400 µg and 800 µg orodispersible dosage form (Examples 4, 5 and 6, respectively) and 2 µg as an i.v. bolus dose (Comparative Example 1). Between the doses there was a washout period of 72 hours. In order to standardize the buccal mucosa before administration of the orodispersible tablet, the subjects were asked to avoid foods, chewing gum etc. Subjects were allowed to brush their teeth in the morning before dosing, but without toothpaste.

Blood Samples

Blood samples for plasma concentration of desmopressin were collected according to the following schedule: pre-dose and 15, 30 and 45 min and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours post-dosing. After intravenous administration additional blood samples were collected 5 and 10 minutes post-dosing.

Assay

The concentration of desmopressin in plasma was determined by a validated RIA method.

Pharmacokinetic Analysis

The concentration of desmopressin in plasma was analyzed for the individual volunteer in each administration group, by use of non-compartmental methods using the com-

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mercially available software WinNonlin™ Pro, ver. 3.2 (Pharsight Corporation, US). A plasma concentration value below limit of quantitation (LOQ) followed by values above LOQ was set at 'LOQ/2' for the NCA analysis and for the descriptive statistics on concentrations. Values below LOQ not followed by values above the LOQ are excluded from the NCA analysis, and set to zero in the descriptive statistics on concentrations.

Results of Pharmacokinetic Analysis

After i.v. administration the mean volume of distribution at steady state (V_{ss}) was 29.7 Dm^3 . The mean clearance was calculated to be 8.5 dm^3/hr and the mean elimination half-life was determined to be 2.8 hours. After oral administration of desmopressin maximum plasma concentrations were observed at 0.5-2.0 hours after dosing. The maximum plasma concentration was 14.25, 30.21 and 65.25 pg/ml after an oral dose of 200, 400 and 800 µg, respectively. After reaching the maximum value desmopressin was eliminated with a mean elimination half-life in the range of 2.8-3.0 hours. The bioavailability was determined to be 0.30% with at 95% confidence interval of 0.23-0.38%.

The pharmacokinetics of desmopressin is linear, when administered as the orodispersible dosage form of Example 4, 5 or 6.

Comparative Example 4

Bioavailability of Desmopressin Administered in Accordance with Comparative Examples 2 and 3

Thirty-six healthy male volunteers (Caucasian, Black and Hispanic) were enrolled in this study, which was designed as an open label, single dose, 3-way crossover study. Each subject was, in a randomized order, administered 200 µg desmopressin as a single 200 µg tablet (Comparative Example 2), 200 µg desmopressin as two 100 µg tablets (Comparative Example 3) and 2 µg as an i.v. bolus dose (Comparative Example 1).

After i.v. administration the mean elimination half-life was determined to be 2.24 hours. After oral administration of desmopressin maximum plasma concentrations were observed at 1.06 hours (2×100 µg) or 1.05 hours (1×200 µg) after dosing. The maximum plasma concentration was 13.2 and 15.0 pg/ml after an oral dose of 2×100 µg and 1×200 µg, respectively. The bioavailability was determined to be 0.13% (2×100 µg) or 0.16% (1×200 µg).

Example 8

Crossover Study Investigating the Antidiuretic Effect of Three Low Doses of Desmopressin

The following Example describes a study showing the antidiuretic effect of three low doses of desmopressin administered via intravenous infusion for 2 hours in over-hydrated, healthy, non-smoking male and female volunteers. Briefly, an open-label, crossover study with 8 healthy, over-hydrated, non-smoking male and female volunteers, age 18-40. The subjects were dosed initially with 0.5 ng/kg dose, then with the 1.0 ng/kg dose and finally the 2.0 ng/kg dose. Pharmacodynamic and pharmacokinetic parameters were evaluated at each dose level. A washout period of two days (48 hours) was observed between dosing.

Eight subjects evaluated in this study, 5 males, and 3 females. Their weights in kilograms were: 85.9, 65, 80.9, 63.3, 72.5, 67.6, 63.5, and 54.5. The mean weight of the 8

subjects was 69.15 kg, which is very close to the standard 70 kg weight estimate upon which the doses and blood levels of desmopressin in this study are based. Subjects were over-hydrated on study day 1 (first day of dosing) by drinking a volume of water equal to 1.5% of body weight and maintained by replacing urine output with water ingestion. Desmopressin of 0.5, 1.0 and 2.0 ng/kg in 100 mL of sterile, physiological saline (0.9%), USP for injection, was used in the study. Three infusions of desmopressin (one at each of the above concentrations) was administered as an I.V. infusion at a constant rate, each 2 hours in duration on days 1, 3 and 5 of the study. Each subject remained in the clinic from one day prior to first dosing to one day after last dosing for a total of 7 days. The first dose was 0.5 ng/kg. Following the end of the desmopressin infusion, subjects voided every 20 minutes and were monitored until 3 consecutive urine collections measured a urine output level exceeding 10 mL/min. At this point over-hydration was discontinued. Urine osmolality was measured 20 minutes before the infusion, at baseline, and with every 20 minute urine collection up to 6 hours after the start of the infusion. Urine-specific gravity was also measured. Plasma/serum sodium and plasma/serum osmolality was measured prior to dosing and at 2, 4, and 6 hours after the start of the infusion. Blood samples for pharmacokinetic determinations were collected predose, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after the start of the infusion. This same procedure was followed for the 1.0 ng/kg and 2.0 ng/kg infusions. On day 6, approximately 24 hours after the third and last desmopressin infusion subjects had an exit physical examination with vital signs, blood and urine laboratory assessments.

Criteria for evaluation in the study included urine output over time, urine osmolality over time, urine-specific gravity over time, and plasma/plasma/serum osmolality and sodium over time. Statistical analysis on the above criteria was performed. The statistical analysis is descriptive and all statistical hypothesis testing was done for exploratory purposes. The following was investigated: duration of action, i.e., time from 'onset' to 'end' action was estimated for each subject using three different levels of osmolality as cut off (150 mOsm/kg, 200 mOsm/kg and 400 mOsm/kg). First, duration of action was defined as the time from onset of action (i.e., the first time after dose administration where urine osmolality was less than 150 mOsm/kg) to end of action (the first subsequent time where urine osmolality was less than 150 mOsm/kg and confirmed at the next interval unless the first subsequent time was the last observation point). The second and third estimation used 200 mOsm/kg and 400 mOsm/kg as cut off levels for 'onset' and 'end' of action, respectively. Subjects with no

'end' of action, with respect to the definition were censored at the time their urinary output returns to baseline (exceeds 10 mL/min) and/or the time where the over-hydration procedure stopped. The overall duration of action was estimated for each dose group using the nonparametric Kaplan-Meier method. The different approaches for estimating duration of action were expected to give lower and upper limits of the true probability, i.e., probability of desmopressin activity as a function of time. Furthermore, the duration of action was presented for each treatment group using the mean, SD, median, minimum and maximum values. The dose-response relationship between duration of action and dose was investigated using an appropriate linear or nonlinear model. Pharmacokinetic parameters were derived from the individual concentration versus time curves of desmopressin, i.e., AUC (area under the plasma concentration time curve to infinity), C_{max} (maximum plasma concentration observed), t_{max} (time of C_{max} after dosing), CL (total systemic clearance), V_z (volume of distribution during the terminal phase), AUC_t (area under the plasma concentration time curve from time zero to time t), λ_z (first order rate constant associated with the terminal (log-linear) portion of the plasma concentration time curve estimated via linear regression of the time vs. log of concentration) and $t_{1/2}$ (terminal half life).

Summary of Results:

All three doses (I.V. infusions) of desmopressin produced a measurable, antidiuretic effects in terms of increased urine concentration (osmolality) and decreased urine output in a dose response fashion. The pharmacodynamic duration of antidiuretic effect also demonstrated a dose response curve with the lowest dose having the shortest duration of effect. The mean peak urine osmolality (mOsm/kg) occurred at the end of the 2 hour infusion for each dose level. Baseline mean urine osmolality was 55.8, 55.8 and 55.6 mOsm/kg for 0.5, 1.0, 2.0 ng/kg doses, respectively. Mean peak urine osmolality was 206.0, 444.7 and 587.2 mOsm/kg at 2 hours for the 0.5, 1.0 and 2.0 ng/kg doses, respectively. The mean nadir urine output (mL/min) also occurred at the end of the 2 hour infusion for each dose level. Baseline mean urine output was 18.6, 16.6 and 16.9 mL/min for the 0.5, 1.0 and 2.0 ng/kg doses, respectively. Mean nadir urine output was 7.1, 1.3, and 0.7 mL/min for the 0.5, 1.0 and 2.0 ng/kg doses, respectively. The duration of antidiuretic effect was approximately 180 minutes for the 0.5 ng/kg dose, 240 to 280 minutes for the 1.0 ng/kg dose and 360 minutes for the 2.0 ng/kg dose. The urine osmolality and output results for each subject and the means for each time period are described in Tables 1-6 and FIGS. 1-9.

TABLE 1

| Subject # | Urine Osmolality (0.5 ng/kg) | | | | | | | | | |
|-----------|------------------------------|------|------|-------|-------|-------|-------|-------|-------|-------|
| | Time (Minutes) | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 |
| 01-001 | 61 | 61 | 63 | 75 | 84 | 91 | 100 | 104 | 93 | 57 |
| 01-002 | 41 | 43 | 46 | 55 | 70 | 83 | 91 | 90 | 79 | 72 |
| 01-003 | 57 | 57 | 65 | 105 | 162 | 228 | 338 | 447 | 363 | 243 |
| 01-004 | 49 | 49 | 97 | 100 | 57 | 56 | 58 | 61 | 59 | 57 |
| 01-005 | 57 | 60 | 95 | 110 | 89 | 83 | 84 | 87 | 80 | 74 |
| 01-006 | 80 | 85 | 115 | 294 | 476 | 621 | 633 | 655 | 670 | 601 |
| 01-007 | 52 | 54 | 56 | 72 | 86 | 95 | 108 | 119 | 87 | 75 |
| 01-008 | 49 | 52 | 48 | 55 | 65 | 69 | 78 | 85 | 75 | 67 |
| Mean | 55.8 | 57.6 | 73.1 | 108.3 | 136.1 | 165.8 | 186.3 | 206.0 | 188.3 | 155.8 |

TABLE 1-continued

| Urine Osmolality (0.5 ng/kg) | | | | | | | | | | |
|------------------------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Subject # | Time (Minutes) | | | | | | | | | |
| | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 78 | * | * | * | * | * | * | * | * | * |
| 01-002 | 66 | * | * | * | * | * | * | * | * | * |
| 01-003 | 177 | 122 | 103 | 93 | 80 | 88 | * | * | * | * |
| 01-004 | 55 | * | * | * | * | * | * | * | * | * |
| 01-005 | 71 | * | * | * | * | * | * | * | * | * |
| 01-006 | 521 | 390 | 327 | 274 | 215 | 250 | 193 | 156 | 133 | 120 |
| 01-007 | 65 | 59 | 57 | * | * | * | * | * | * | * |
| 01-008 | 61 | 60 | 59 | 58 | * | * | * | * | * | * |
| Mean | 136.8 | 157.8 | 136.5 | 141.7 | 147.5 | 169.0 | 193.0 | 156.0 | 133.0 | 120.0 |

TABLE 2

| Urine Osmolality (1.0 ng/kg) | | | | | | | | | | |
|------------------------------|----------------|------|------|-------|-------|-------|-------|-------|-------|-------|
| Subject # | Time (Minutes) | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 |
| 01-001 | 58 | 59 | 65 | 108 | 281 | 305 | 480 | * | 435 | 132 |
| 01-002 | 46 | 44 | 53 | 91 | 168 | 222 | 315 | 414 | 324 | 230 |
| 01-003 | 48 | 51 | 60 | 178 | 406 | 402 | 506 | 595 | 618 | * |
| 01-004 | 48 | 49 | 52 | 68 | 92 | 135 | 180 | 219 | 156 | 105 |
| 01-005 | 68 | 68 | 73 | 106 | 166 | 235 | 260 | 312 | 204 | 142 |
| 01-006 | 82 | 82 | 124 | 585 | 614 | 638 | 708 | 747 | 736 | 733 |
| 01-007 | 47 | 47 | 53 | 100 | 175 | * | 267 | 381 | * | 228 |
| 01-008 | 49 | 52 | 57 | 100 | 173 | * | 288 | * | * | * |
| Mean | 55.8 | 56.5 | 67.1 | 167.0 | 259.4 | 322.8 | 375.5 | 444.7 | 412.2 | 261.7 |

| Subject # | Time (Minutes) | | | | | | | | | |
|-----------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 150 | 160 | 71 | 60 | * | * | * | * | * | * |
| 01-002 | 171 | 127 | 116 | 104 | 99 | 98 | 82 | 68 | 62 | * |
| 01-003 | 588 | 374 | 322 | 221 | 162 | 148 | 111 | 96 | * | * |
| 01-004 | 85 | 71 | 71 | 67 | * | * | * | * | * | * |
| 01-005 | 109 | 94 | 88 | 83 | 75 | * | * | * | * | * |
| 01-006 | 771 | 694 | * | 747 | 606 | 655 | 687 | 546 | 458 | 374 |
| 01-007 | 122 | 96 | 86 | 81 | 69 | 69 | 57 | 53 | 47 | 44 |
| 01-008 | 251 | 114 | 96 | 90 | 80 | * | 73 | 61 | 55 | 51 |
| Mean | 280.9 | 216.3 | 121.4 | 181.6 | 181.8 | 242.5 | 202.0 | 164.8 | 155.5 | 156.3 |

TABLE 3

| Urine Osmolality (2.0 ng/kg) | | | | | | | | | | |
|------------------------------|----------------|------|------|-------|-------|-------|-------|-------|-------|-------|
| Subject # | Time (Minutes) | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 |
| 01-001 | 63 | 63 | 88 | 373 | * | 526 | * | * | 585 | * |
| 01-002 | 40 | 40 | 46 | 149 | 251 | * | 492 | * | 601 | 533 |
| 01-003 | 51 | 52 | 73 | 337 | 401 | * | * | 568 | * | * |
| 01-004 | 45 | 48 | 50 | 146 | 298 | 390 | 442 | 461 | 478 | 439 |
| 01-005 | 78 | 73 | 119 | 293 | 499 | 501 | 421 | 564 | 492 | 492 |
| 01-006 | 71 | 73 | 108 | 604 | 626 | 698 | 748 | 769 | 771 | 727 |
| 01-007 | 45 | 45 | 60 | * | * | * | * | * | * | 509 |
| 01-008 | 52 | 54 | 61 | 208 | 385 | 465 | 525 | 574 | 533 | 508 |
| Mean | 55.6 | 56.0 | 75.6 | 301.4 | 410.0 | 516.0 | 525.6 | 587.2 | 576.7 | 534.7 |

| Subject # | Time (Minutes) | | | | | | | | | |
|-----------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 571 | 623 | 482 | * | 458 | 384 | 346 | 146 | 73 | 76 |
| 01-002 | 538 | 489 | 385 | 348 | 252 | 244 | 173 | 116 | 87 | 76 |
| 01-003 | 568 | * | 541 | 559 | 477 | 476 | 380 | 267 | 179 | 134 |
| 01-004 | 357 | 250 | 195 | 139 | 110 | 112 | 97 | 73 | 66 | 60 |

TABLE 3-continued

| Urine Osmolality (2.0 ng/kg) | | | | | | | | | | |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 01-005 | 390 | 387 | 352 | 267 | 195 | 178 | 154 | 104 | 98 | 87 |
| 01-006 | 733 | 676 | 677 | 668 | 640 | 665 | 648 | 585 | 577 | 547 |
| 01-007 | * | * | * | 666 | * | * | * | 255 | 100 | 79 |
| 01-008 | 583 | 542 | * | 539 | * | * | 473 | * | 204 | 91 |
| Mean | 534.3 | 494.5 | 438.7 | 455.1 | 355.3 | 343.2 | 324.4 | 220.9 | 173.0 | 143.8 |

TABLE 4

| Urine Output (0.5 ng/kg) | | | | | | | | | | |
|--------------------------|----------------|------|------|------|------|------|------|------|------|------|
| Subject # | Time (Minutes) | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 |
| 01-001 | 20 | 18.8 | 16.7 | 14 | 10.9 | 10 | 9.1 | 5 | 17.6 | 15 |
| 01-002 | 17 | 16.5 | 16.4 | 13.8 | 10.8 | 9.3 | 8.8 | 8.3 | 11.8 | 10.5 |
| 01-003 | 18.8 | 17.4 | 16.7 | 9 | 5.9 | 4.1 | 2.4 | 2 | 2 | 3 |
| 01-004 | 26 | 22.3 | 8.9 | 10.5 | 16.4 | 16.5 | 16.5 | 15.2 | 16 | 17 |
| 01-005 | 19.5 | 20 | 11.8 | 9 | 12.5 | 10 | 9.5 | 10.9 | 12.5 | 13 |
| 01-006 | 15.9 | 13 | 8.8 | 3.1 | 1.4 | 1.1 | 1.1 | 0.9 | 1.2 | 1.8 |
| 01-007 | 16.1 | 25.8 | 14 | 13.3 | 9.6 | 9.2 | 7 | 8 | 8.7 | 10 |
| 01-008 | 15.5 | 13.3 | 12.2 | 10 | 9.1 | 8.1 | 7.5 | 6.5 | 7.1 | 7.6 |
| Mean | 18.6 | 18.4 | 13.2 | 10.3 | 9.6 | 8.5 | 7.7 | 7.1 | 9.6 | 9.7 |

| Subject # | Time (Minutes) | | | | | | | | | |
|-----------|----------------|------|------|------|------|-----|-----|-----|-----|-----|
| | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 13.6 | * | * | * | * | * | * | * | * | * |
| 01-002 | 13.6 | * | * | * | * | * | * | * | * | * |
| 01-003 | 6.4 | 8.3 | 8.5 | 11.4 | 15.6 | 15 | * | * | * | * |
| 01-004 | 17.1 | * | * | * | * | * | * | * | * | * |
| 01-005 | 15.7 | * | * | * | * | * | * | * | * | * |
| 01-006 | 2 | 2.2 | 3.8 | 4.2 | 3.8 | 3.2 | 4.1 | 5 | 6.4 | 7.3 |
| 01-007 | 12.2 | 17.9 | 16.7 | * | * | * | * | * | * | * |
| 01-008 | 8.2 | 13.3 | 12.5 | 10.5 | * | * | * | * | * | * |
| Mean | 11.1 | 10.4 | 10.4 | 8.7 | 9.7 | 9.1 | 4.1 | 5.0 | 6.4 | 7.3 |

TABLE 5

| Urine Output (1.0 ng/kg) | | | | | | | | | | | | | | | | | | | | |
|--------------------------|----------------|------|------|------|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|-----|------|------|------|------|
| Subject # | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 16.8 | 17.4 | 10.4 | 7.6 | 1.9 | 2.4 | 1.1 | 0 | 0.8 | 2.4 | 2.6 | 13.2 | 15.9 | 12.9 | * | * | * | * | * | * |
| 01-002 | 17.1 | 18 | 15.6 | 8.4 | 4.2 | 3.5 | 2.2 | 1.6 | 2.6 | 3.1 | 4.5 | 6 | 5.6 | 6.3 | 7.5 | 7.7 | 10.5 | 10.1 | 14.2 | |
| 01-003 | 18.5 | 18 | 14 | 4 | 1.4 | 1.6 | 0.9 | 0.7 | 0.8 | 0 | 1.7 | 1.6 | 2.1 | 3.9 | 5.8 | 5.7 | 8.8 | 10.6 | 13.9 | 15.5 |
| 01-004 | 22 | 19.3 | 17.1 | 12.5 | 8.5 | 4.8 | 3.7 | 3.2 | 5 | 8.1 | 10 | 12.4 | 11.6 | 14.1 | * | * | * | * | * | * |
| 01-005 | 19.5 | 20 | 15.2 | 9.9 | 5.7 | 3 | 3 | 2.6 | 4.3 | 5.3 | 7.9 | 8.8 | 11.8 | 11.8 | 11.7 | * | * | * | * | * |
| 01-006 | 13 | 12.4 | 7.2 | 1.2 | 0.8 | 0.6 | 0.7 | 0.6 | 0.7 | 0.7 | 0.6 | 0 | 1.1 | 0.8 | 0.6 | 0.9 | 1.1 | 1 | 1 | 1.9 |
| 01-007 | 16 | 15.9 | 13.2 | 6.5 | 3.7 | 0 | 4.3 | 1.3 | 0 | 5.8 | 5.1 | 6.9 | 7.3 | 7.7 | 9 | 9.3 | 8.9 | 11.6 | 16 | 15.7 |
| 01-008 | 10.2 | 12.5 | 11.2 | 5.7 | 3.5 | 0 | 3.7 | 0 | 0 | 0 | 7.1 | 4.3 | 4.2 | 5.5 | 4.7 | 0 | 11.5 | 6.7 | 8 | 8.6 |
| Mean | 16.6 | 16.7 | 13.0 | 7.0 | 3.7 | 2.0 | 2.5 | 1.3 | 1.8 | 3.2 | 5.0 | 6.7 | 7.3 | 7.9 | 6.6 | 4.7 | 8.1 | 8.0 | 10.6 | 10.4 |

TABLE 6

| Urine Output (2.0 ng/kg) | | | | | | | | | | | | | | | | | | | | |
|--------------------------|----------------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|
| Subject # | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-001 | 14.5 | 16 | 9.3 | 1.2 | 0 | 1.5 | 0 | 0 | 0.9 | 0 | 1 | 1.9 | 0.5 | 2.7 | 1.9 | 1.6 | 2.1 | 13.6 | 11.2 | 11.4 |
| 01-002 | 19.5 | 20 | 14.4 | 3 | 2.7 | 0 | 2.3 | 0 | 3.3 | 0.9 | 0.8 | 0.9 | 1.1 | 1.8 | 2.8 | 2.6 | 3.9 | 6.2 | 9.5 | 12.9 |
| 01-003 | 18.5 | 18.3 | 10.8 | 1.6 | 1.8 | 0 | 0 | 2.7 | 0 | 0 | 2.8 | 0 | 2.4 | 1.4 | 2 | 1.9 | 2 | 4.6 | 6.9 | 8.6 |
| 01-004 | 22 | 20.5 | 14.4 | 5.1 | 1.8 | 1.5 | 0.8 | 1 | 1.3 | 1.5 | 2 | 3.1 | 4.1 | 6 | 8.3 | 8.4 | 9.4 | 11.6 | 14.3 | 13.9 |
| 01-005 | 18 | 17.6 | 9.2 | 3.5 | 1.7 | 1.4 | 1.4 | 1 | 1.2 | 1.2 | 1.6 | 1.6 | 1.7 | 2.6 | 3.8 | 4.9 | 5.7 | 8.3 | 10.3 | 12.5 |
| 01-006 | 14 | 12.9 | 6.5 | 0.8 | 0.7 | 0.4 | 0.5 | 0.4 | 0.7 | 0.5 | 0.7 | 0.6 | 0.7 | 0.7 | 0.7 | 0.7 | 1 | 0.9 | 1 | 1.2 |

TABLE 6-continued

| Subject # | Urine Output (2.0 ng/kg) | | | | | | | | | | | | | | | | | | | |
|-----------|--------------------------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | Time (Minutes) | | | | | | | | | | | | | | | | | | | |
| | -20 | 0 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 260 | 280 | 300 | 320 | 340 | 360 |
| 01-007 | 14.5 | 13.2 | 9.1 | 0 | 0 | 0 | 0 | 0 | 0 | 4.6 | 0 | 0 | 0 | 1.7 | 0 | 0 | 0 | 7.3 | 6.5 | 9.1 |
| 01-008 | 14.5 | 13.1 | 10 | 2.9 | 1.6 | 0.5 | 0.9 | 0.4 | 0.4 | 0.5 | 0.4 | 0.4 | 0 | 1 | 0 | 0 | 1.4 | 0 | 3.5 | 4.6 |
| Mean | 16.9 | 16.5 | 10.5 | 2.3 | 1.3 | 0.7 | 0.7 | 0.7 | 1.0 | 1.2 | 1.2 | 1.1 | 1.3 | 2.2 | 2.4 | 2.5 | 3.2 | 6.6 | 7.9 | 9.3 |

As shown in Tables 1-6 and FIGS. 1-9, low doses of desmopressin administered as I.V. infusions over 2 hours produced significant antidiuretic effects in over-hydrated, normal subjects in a dose response fashion. These doses and calculated plasma/serum concentrations of desmopressin were far lower than the current labeled recommendations and current clinical practice by a factor of more than one order of magnitude. The pharmacodynamic duration of action was also proportional to the dose with the 1.0 and 2.0 ng/kg doses providing durations of 4 to 6 hours. This may be adequate to produce the desired therapeutic effects for existing and potential new clinical indications for desmopressin. Safety and tolerability were excellent.

The results of this study confirm the low-dose hypothesis for desmopressin and provide an empirical basis for further clinical studies in patients to evaluate low doses of desmopressin for such conditions as primary nocturnal enuresis, adult nocturia, incontinence and central diabetes insipidus.

The therapeutic effectiveness of desmopressin for all these clinical indications is based on desmopressin's antidiuretic pharmacological effect which results in production of smaller volumes of more concentrated urine. For patients with central diabetes insipidus, the pituitary gland produces little or no vasopressin, the natural antidiuretic hormone. This deficiency results in large volumes of very dilute urine being produced which can lead to dehydration and serious metabolic abnormalities unless the patient consumes very large volumes of water. Desmopressin replaces the deficient vasopressin and restores normal urine concentration and volume in these patients. In patients with primary nocturnal enuresis (bed wetting), the antidiuretic effect of desmopressin decreases urine volume at night, lowering the amount of urine which the urinary bladder must retain and, thereby decreasing or eliminating occurrences of enuresis.

In patients with adult nocturia, there is either polycoma (production of large amounts of urine) at night, low bladder capacity or increased bladder sensitivity to urine volume. Under all these circumstances, the bladder's threshold for urine retention is exceeded during the night, often several times, resulting in neurological signals for voiding. This awakens the patient in order to void. Desmopressin's antidiuretic effect decreases urine production at night delaying the time when the voiding threshold is exceeded resulting in a longer sleep period before voiding and decreasing the number of nocturnal voids.

In patients with incontinence of various types (stress, urge, etc.) often related to urinary bladder abnormalities from surgery, childbirth, and aging, the bladder is unable to retain even normal volumes of urine. The volume threshold for voiding is low and there is a high risk of involuntary voiding (incontinence). Desmopressin's antidiuretic effect decreases urine production allowing for voiding postponement because there is a delay in crossing the abnormally low volume threshold for voiding in these patients.

In all the above clinical indications, or medical uses of desmopressin, its antidiuretic pharmacological effect resulting in decreased production of more concentrated urine is the mechanism of therapeutic effectiveness. This clinical study demonstrates that desmopressin can produce this essential antidiuretic effect at much lower doses and lower blood concentrations than previously thought. Therefore, lower doses and concentrations of desmopressin may be used for treating patients with all of the above conditions.

While the invention has been described above with reference to specific embodiments thereof, it is apparent that many changes, modifications, and variations can be made without departing from the inventive concept disclosed herein. Accordingly, it is intended to embrace all such changes, modifications, and variations that fall within the spirit and broad scope of the appended claims. All patent applications, patents, and other publications cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method for inducing voiding postponement in a patient while reducing the risk that the patient develops hyponatremia comprising delivering to the bloodstream of the patient an amount of desmopressin no more than about 2 ng/kg by intranasal, transdermal, intradermal, transmucosal, or conjunctival administration, said amount being therapeutically effective to produce an antidiuretic effect lasting for no more than between about 4 and about 6 hours.

2. The method of claim 1 comprising delivering to the bloodstream of the patient an amount of desmopressin no more than about 1 ng/kg.

3. The method of claim 1 further comprising advising a patient that fluid intake should be restricted after administration.

4. The method of claim 1 further comprising advising the patient that no water should be taken after administration.

5. The method of claim 1 comprising administering desmopressin to a patient suffering from nocturia, primary nocturnal enuresis (PNE), or incontinence.

6. The method of claim 1 wherein the method produces a plasma/serum desmopressin concentration in the patient of a maximum of no more than about 10 pg/ml.

7. The method of claim 1 wherein the method produces a plasma/serum desmopressin concentration in the patient of a maximum of no more than about 5 pg/ml.

8. A method for inducing voiding postponement comprising administering to a patient an amount of desmopressin sufficient to produce in the patient a urine osmolality ranging above about 300 mOsm/kg for less than about 5 hours after administration.

9. The method of claim 1 or 8 comprising administering the desmopressin by intranasal administration.

10. The method of claim 1 or 8 comprising administering the desmopressin by transdermal administration.

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11. The method of claim 1 or 8 comprising administering the desmopressin by intradermal administration.

12. The method of claim 1 or 8 comprising administering the desmopressin by transmucosal administration.

13. The method of claim 1 or 8 comprising administering the desmopressin by conjunctival administration.

14. The method of claim 1 or 8 comprising administering to the patient between 100 and 2000 ng (0.1 µg to 2 µg) desmopressin.

15. The method of claim 8 wherein the method produces a plasma/serum desmopressin concentration in the patient no more than about 10 pg/ml.

16. The method of claim 8 wherein the method produces a plasma/serum desmopressin concentration in the patient no more than about 5 pg/ml.

17. The method of claim 8 comprising delivering to the bloodstream of the patient no more than about 2 ng/kg desmopressin.

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18. The method of claim 8 comprising delivering desmopressin to the bloodstream of a patient suffering from nocturia, PNE, or incontinence.

19. A method for inducing voiding postponement in a patient while reducing the risk that the patient develops hyponatremia comprising delivering to the bloodstream of the patient via transdermal, intradermal, transmucosal, or conjunctival administration no more than about 1 ng/kg desmopressin to produce an antidiuretic effect for no more than about four to about six hours.

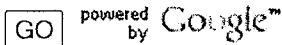
20. A method for inducing voiding postponement in a patient while reducing the risk that the patient develops hyponatremia comprising delivering to the bloodstream of the patient via intranasal administration no more than about 2 ng/kg of desmopressin so as to produce an antidiuretic effect.

21. The method of claim 20 comprising delivering to the bloodstream of the patient no more than about 1 ng/kg desmopressin.

* * * * *

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Information for Healthcare Professionals

Desmopressin Acetate (marketed as DDAVP Nasal Spray, DDAVP Rhinal Tube, DDAVP, DDVP, Minirin, and Stimate Nasal Spray)



FDA ALERT [12/4/2007]: FDA has requested the manufacturers update the prescribing information for desmopressin to include important new information about severe hyponatremia and seizures.

Certain patients taking desmopressin are at risk for developing severe hyponatremia that can result in seizures and death. Children treated with desmopressin *intranasal* formulations for primary nocturnal enuresis (PNE) are particularly susceptible to severe hyponatremia and seizures. As such, desmopressin *intranasal* formulations are no longer indicated for the treatment of primary nocturnal enuresis and should not be used in hyponatremic patients or patients with a history of hyponatremia. PNE treatment with desmopressin *tablets* should be interrupted during acute illnesses that may lead to fluid and/or electrolyte imbalance. *All* desmopressin formulations should be used cautiously in patients at risk for water intoxication with hyponatremia.

This information reflects FDA's current analysis of data available to FDA concerning this drug. FDA intends to update this sheet when additional information or analyses become available.

To report any unexpected adverse or serious events associated with the use of this drug, please contact the FDA MedWatch program and complete a form on line at <http://www.fda.gov/medwatch/report/hcp.htm> or report by fax to 1-800-FDA-0178, by mail using the postage-paid address form provided on line, or by telephone to 1-800-FDA-1088.

FDA has requested that the prescribing information for all desmopressin products be updated with new information about the risk for hyponatremia and how to safely use desmopressin.

- The **intranasal formulations** are no longer indicated for treating primary nocturnal enuresis. The new information about the risk for hyponatremia can be found in DDAVP's INDICATION AND USAGE, CONTRAINDICATIONS, WARNINGS, PRECAUTIONS, DOSAGE and ADMINISTRATION, and HOW SUPPLIED sections of the prescribing information and the PATIENT INSTRUCTION GUIDE. Label  The other desmopressin product's labeling will be similarly updated. All other approved indications of the individual intranasal formulations still remain.
- For DDAVP's **desmopressin tablets, rhinal tube, and injections** Label  the new information about the risk for hyponatremia can be found in the CONTRAINDICATIONS,

WARNINGS, and DOSAGE and ADMINISTRATION sections of the prescribing information.

Recommendations and Considerations

- **Desmopressin *intranasal* formulations are no longer indicated for the treatment of primary nocturnal enuresis due to serious hyponatremia that may result in seizures and death.** Doctors should consider other options for managing this condition.
- **Desmopressin *tablets*:**
 - **Treatment for primary nocturnal enuresis should be interrupted during episodes of fluid and/or electrolyte imbalance**, such as fever, recurrent vomiting or diarrhea, vigorous exercise, or other conditions associated with increased water consumption.
 - **Fluid intake should be restricted from 1 hour before to 8 hours after administration of desmopressin tablets.**
- ***All* desmopressin formulations should be used cautiously in patients with habitual or psychogenic polydipsia or in patients who are taking drugs that may cause them to drink more fluids, such as tricyclic antidepressants and selective serotonin re-uptake inhibitors (SSRIs).** Patients taking desmopressin and consuming excessive fluids are at higher risk of developing hyponatremia.

Information for the patient: *Physicians who prescribe desmopressin should discuss with their patients:*

Desmopressin works by limiting the amount of water that is eliminated in the urine. A healthy body needs to maintain a balance of water and salt ("sodium"). If sodium levels fall too much ("hyponatremia"), a person may have seizures and, in extreme cases, may die. That is why it is important to monitor your or your child's water intake. A person's chance of water and sodium imbalance is increased

- if they are taking certain medicines such as antidepressants, painkillers, and medicines to treat seizures that may make the mouth dry
- during hot weather or following strenuous exercise that may make them thirsty
- if they are sick and have severe vomiting and diarrhea, fever, the flu, or severe cold

Therefore, if you or child are prescribed desmopressin, it is important that you

- tell your doctor about other medicines you or your child are taking
- tell your doctor if you or your child has a history of hyponatremia
- supervise the use of desmopressin in your child if it is administered in the nose for the remaining indications
- restrict fluid intake from 1 hour before to 8 hours after taking desmopressin tablets
- promptly contact your doctor if your or your child's water intake changes
- promptly contact your doctor if symptoms of hyponatremia occur, such as nausea, vomiting, fatigue, muscle cramps or weakness

Background Information and Data

Desmopressin is a synthetic analogue of vasopressin, an antidiuretic hormone that prevents excessive water loss in the urine. Desmopressin in combination with excessive fluid consumption can result in hyponatremia, an imbalance between intracellular and extracellular sodium. This imbalance can lead to seizures, brain swelling, and death.

FDA has reviewed 61 postmarketing cases of hyponatremic-related seizures associated with the use of desmopressin. Fifty-five cases reported sodium levels ranging from 104 to 130 mEq/L during the seizure event. In two cases, the patients died. Both patients experienced hyponatremia and seizures but the direct contribution of desmopressin to the deaths is unclear. Thirty-six cases were associated with intranasal formulations, of which 25 cases occurred in pediatric patients (<17 years old). The most commonly reported indication of use in these 25 pediatric cases was nocturnal enuresis. Thirty-nine of the 61 cases were associated with at least one concomitant drug or disease that is also associated with hyponatremia and/or seizures.

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EXAMINER

KOSAR, ANDREW D

ART UNIT

PAPER NUMBER

1654

DATE MAILED: 04/07/2008

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
|-----------------|-------------|----------------------|---------------------|------------------|

11/744,615

05/04/2007

Seymour H. Fein

SER-001D1

9172

TITLE OF INVENTION: PHARMACEUTICAL COMPOSITIONS INCLUDING LOW DOSAGES OF DESMOPRESSIN

| APPLN. TYPE | SMALL ENTITY | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|----------------|--------------|---------------|---------------------|----------------------|------------------|------------|
| nonprovisional | YES | \$720 | \$300 | \$0 | \$1020 | 07/07/2008 |

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:**I. Review the SMALL ENTITY status shown above.**

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

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III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Applicant's representative, Edmund Pitcher, on March 26, 2008.

Response to Amendments/Argument

Applicant's arguments and amendments filed February 1, 2008 are acknowledged. Any rejection and/or objection not specifically addressed is herein withdrawn. The examiner notes that the claim amendments were not provided in proper format, however in the interest of compact prosecution and for clarity of the record, the allowable claims have been presented below in their entirety.

The declaration of Dr. Berl under 37 CFR 1.132 filed January 4, 2008 has been considered, and in view of the amendments to the claims, the affidavit is sufficient to overcome the rejections of record under 35 USC 102.

Examiner's Amendment

The application has been amended as follows:

REPLACE claim 19-27, 29-31, 33 and 34 with the following:

19. A method of treating nocturia, primary nocturnal enuresis, or incontinence, or for inducing voiding postponement, said method comprising administering to a patient in need thereof a pharmaceutical composition comprising a dose of desmopressin sufficient to achieve a

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maximum desmopressin plasma/serum concentration no greater than 10 pg/ml and maintaining the concentration within the range of about 0.5 pg/ml and 10 pg/ml for about four to six hours.

20. The method of claim 19, comprising administering said composition by transmucosal, transdermal, or intradermal delivery.
21. The method of claim 19, comprising treating nocturia.
22. The method of claim 19, comprising administering said composition by intravenous delivery.
23. The method of claim 19, comprising administering said composition by subcutaneous delivery.
24. The method of claim 19, comprising administering said composition by transmucosal delivery.
25. The method of claim 19, comprising administering said composition by transdermal delivery.
26. The method of claim 19, comprising administering said composition by intradermal delivery.
27. The method of claim 19, wherein the desmopressin plasma/serum concentration is maintained at a level no greater than about 5 pg/ml.
28. (cancelled)
29. A method for inducing an antidiuretic effect in a patient comprising the step of administering to a patient a pharmaceutical composition comprising desmopressin by transmucosal, transdermal, or intradermal delivery in an amount and for a time sufficient to establish a maximum serum/plasma desmopressin concentration no greater than 10 pg/ml.

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30. The method of claim 29, wherein said patient is suffering from incontinence, primary nocturnal enuresis (PNE), or nocturia.

31. The method of claim 29, wherein said desmopressin pharmaceutical composition is administered in an amount and for a time sufficient to establish a serum/plasma desmopressin concentration no greater than about 5 pg/ml.

32. (cancelled)

33. A method for treating a patient suffering from nocturia comprising administering to a patient a pharmaceutical composition comprising desmopressin by transmucosal, transdermal, or intradermal delivery in an amount and for a time sufficient to establish a maximum serum/plasma desmopressin concentration greater than 0.1 pg/ml and less than 10 pg/ml.

34. The method of claim 29, wherein the patient is a human or other mammalian subject.

ADD NEW claim 35:

35. The method of claim 33, wherein said concentration is maintained greater than 0.1 pg/ml for a time greater than 4 hours.

Reasons for Allowance

The following is an examiner's statement of reasons for allowance:

As amended above, the claims are distinguished over the art, as the art did not recognize achieving a Cmax of 10 pg/ml or less. For example, BENGTSSON (US Patent 5,763,398; PTO-1449, 5/4/07) teaches achieving a Cmax of approximately 400 pg/ml, however there is no

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teaching or suggesting in Bengtsson, or in combination with any other art of record, to lower the C_{max} to 10 pg/ml.

Furthermore, the independent claims have been amended to reflect the C_{max} of desmopressin is no greater than 10 pg/ml, which finds support in the specification, particularly at paragraphs [0138] and [0141] of the parent Application (paragraphs [0130] and [0133] of the instant specification). The examiner notes that previously such limitations presented in the claims were considered, and rejected as, new matter. However, upon further review, the examiner reverses his opinion and finds sufficient evidence and support for the limitations now presented and placing the claims in condition for allowance. The specification discusses producing C_{max} in a linear manner for doses p.o. of 200, 400 and 800 µg and discusses C_{max} values for i.v. administration. Taken together with these examples, along with the discussion of the necessity for ‘low dosages’, one would reasonably find support under 112 1st paragraph for a C_{max} no greater than 10 pg/ml.

With regards to the enablement/written description rejections, the examiner finds the current amended claims are supported by the disclosure sufficient to withdraw the rejections. The claims are no longer drawn to treating any/all diseases, but rather to a select group of diseases with a single compound, and it would not pose an undue burden to determine what dosage/dosage form would be necessary to achieve the requisite desmopressin C_{max} as in the claims, particularly since the examples show a linear correlation between dose and C_{max}. Achieving the C_{max} would amount to nothing more than routine optimization.

Conclusion

Claims 19-27, 29-31 and 33-35 are allowed.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Andrew D. Kosar whose telephone number is (571)272-0913. The examiner can normally be reached on Monday - Friday 08:00 - 16:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia J. Tsang can be reached on (571)272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Andrew D Kosar/
Primary Examiner, Art Unit 1654



US005135480A

United States Patent [19]**Bannon et al.**[11] **Patent Number:** **5,135,480**[45] **Date of Patent:** **Aug. 4, 1992**[54] **TRANSDERMAL DRUG DELIVERY DEVICE**

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[21] **Appl. No.:** **617,945**

[22] **Filed:** **Nov. 21, 1990**

Related U.S. Application Data

[63] Continuation of Ser. No. 71,755, Jul. 9, 1987, abandoned.

[30] **Foreign Application Priority Data**

Jul. 10, 1986 [IE] Ireland 1854/86

[51] **Int. Cl.⁵** **A61N 1/30**

[52] **U.S. Cl.** **604/20; 128/639;**
128/644; 128/799

[58] **Field of Search** **128/639-644,**
128/635, 798, 799, 802, 803; 604/20

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Primary Examiner—Lee S. Cohen

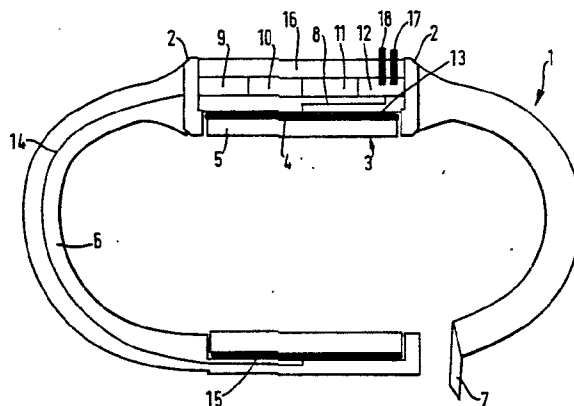
Assistant Examiner—J. P. Lacyk

Attorney, Agent, or Firm—Marla J. Church

[57]

ABSTRACT

A transdermal device having a detachably mounted electrode with a first surface adapted for contact with human skin and through which a drug substance contained in the electrode passes to the skin under the influence of an iontophoretic or electro-osmotic force and a second surface which is electrically conducting, the electrode has a surface area in contact with the skin, in use, in the range 0.1 to 30 cm² and a drug dissolved or dispersed in a hydrophilic medium at a concentration in the range 0.1 to 15% (w/v) based on the hydrophilic medium.

16 Claims, 7 Drawing Sheets

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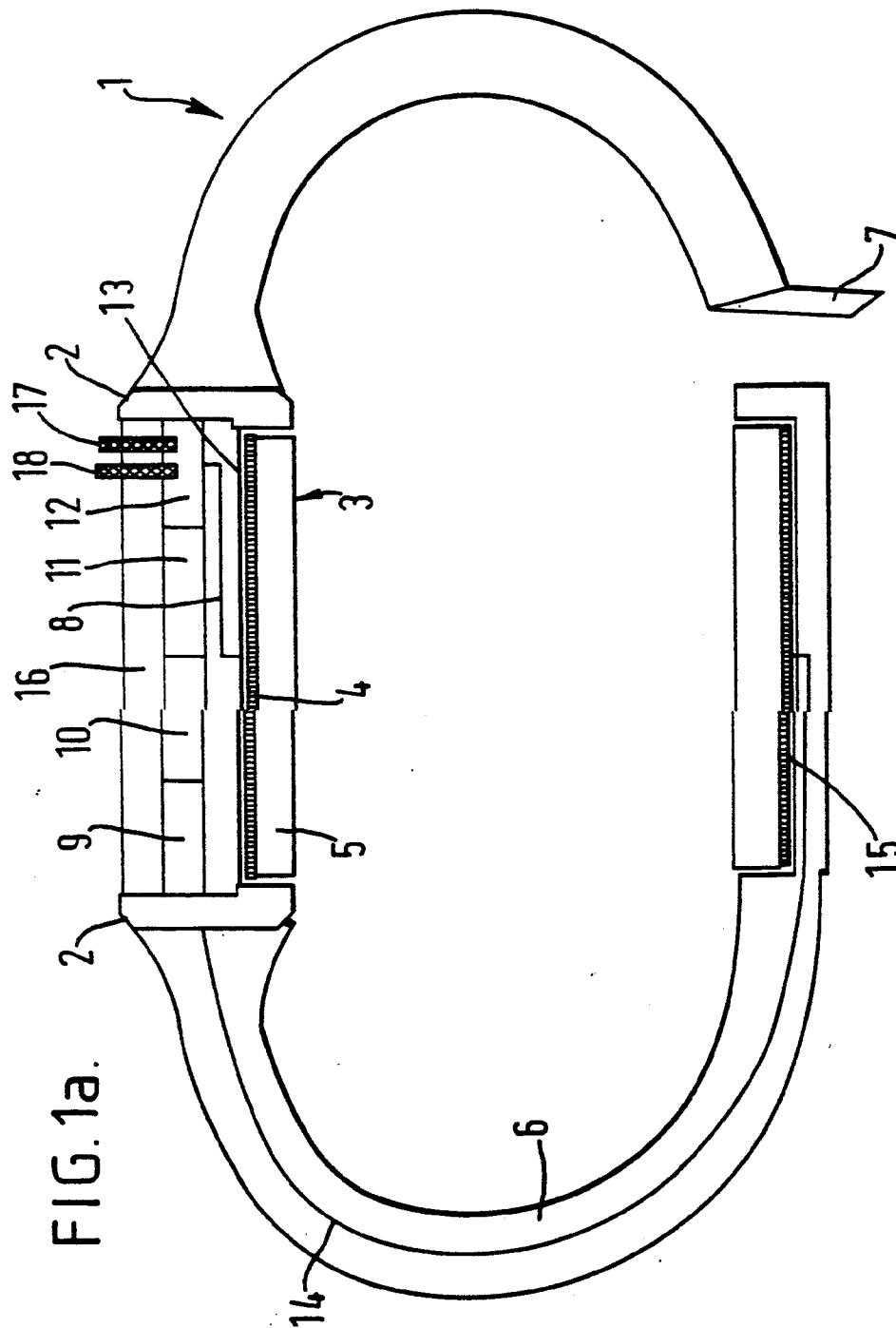


FIG. 1b.

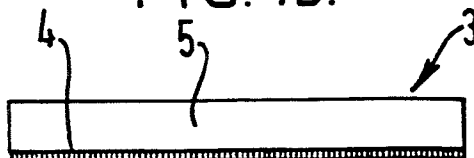


FIG. 2.

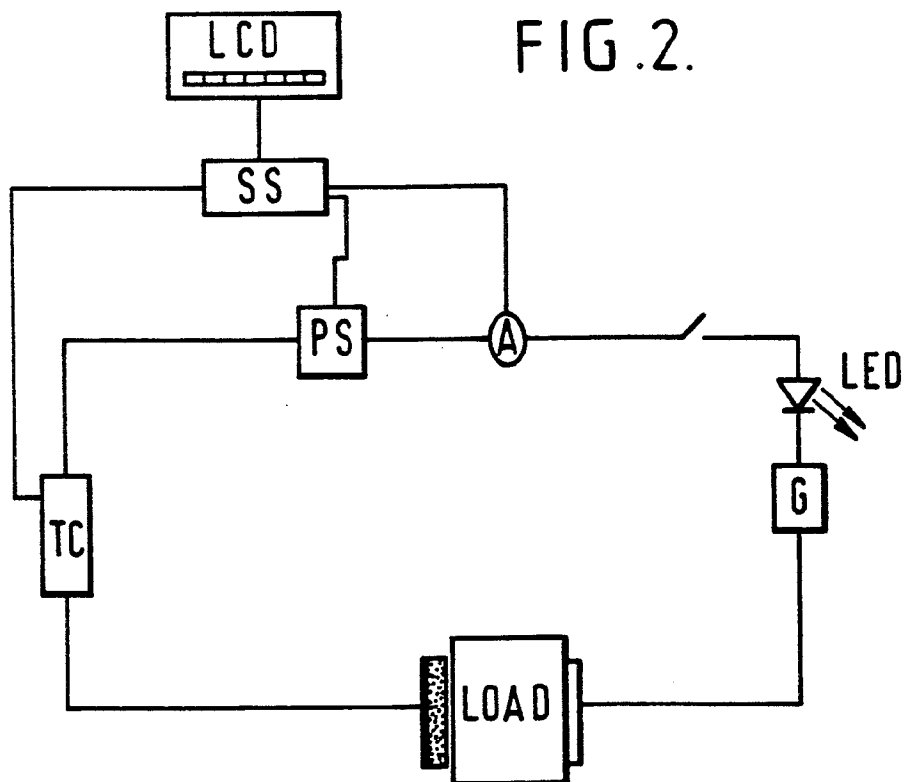


FIG. 3.

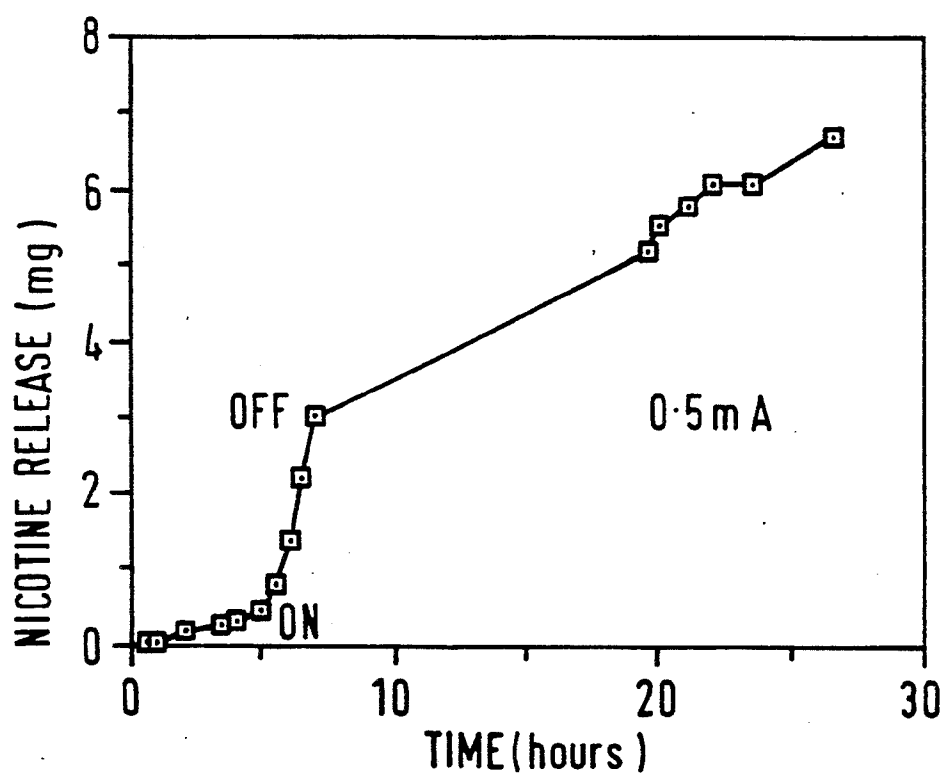


FIG. 4.

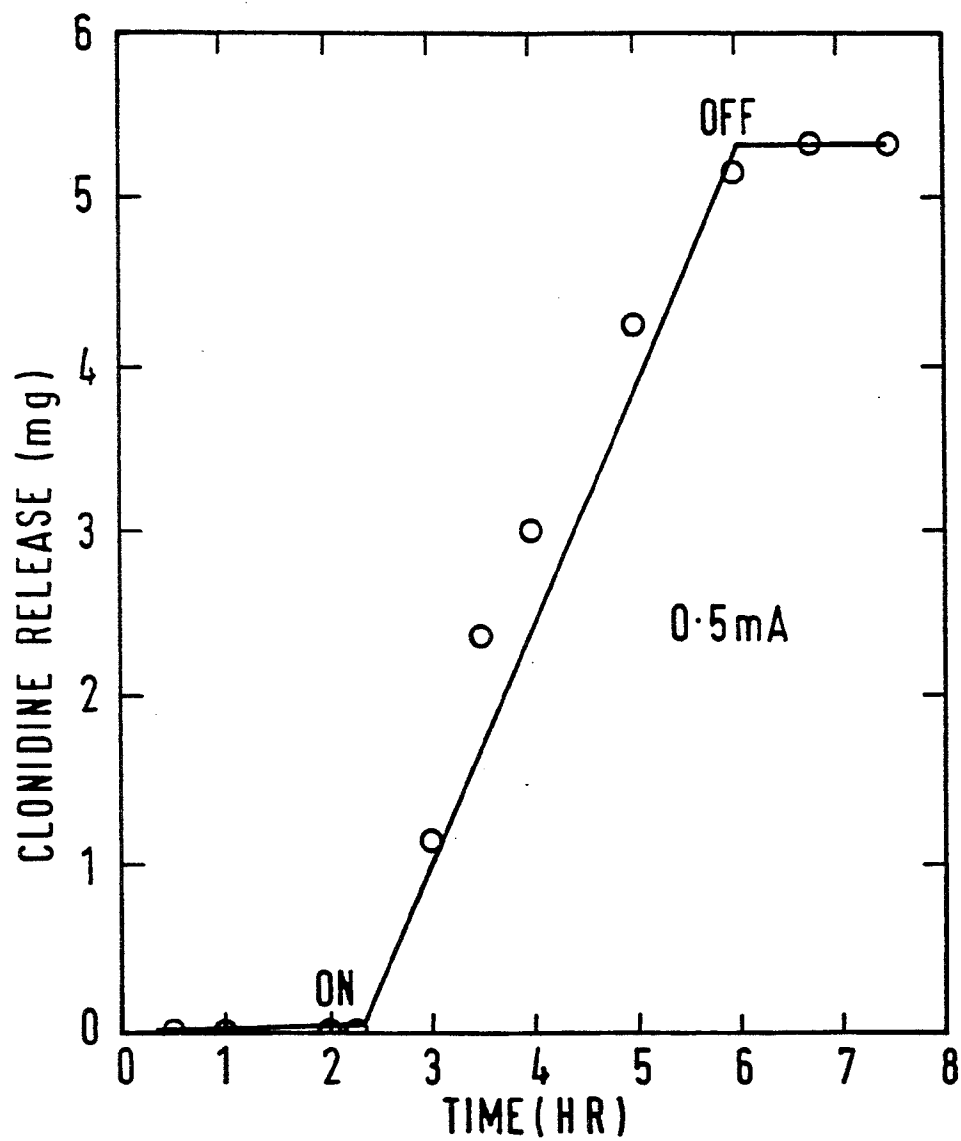
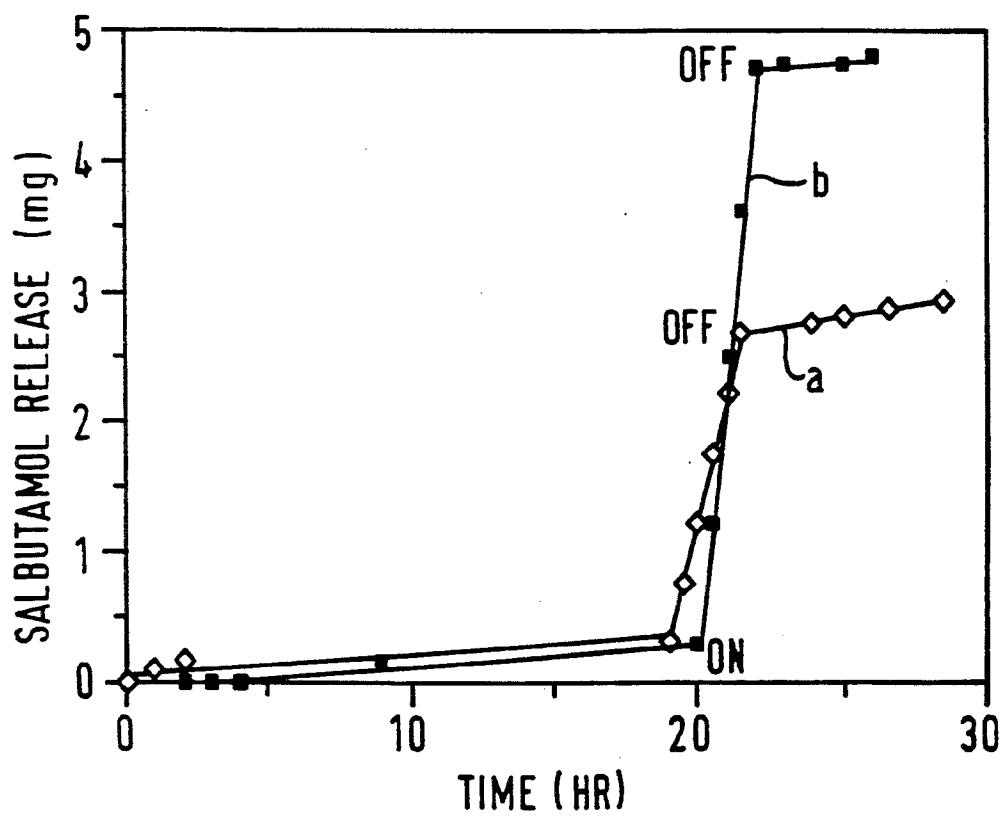


FIG. 5.



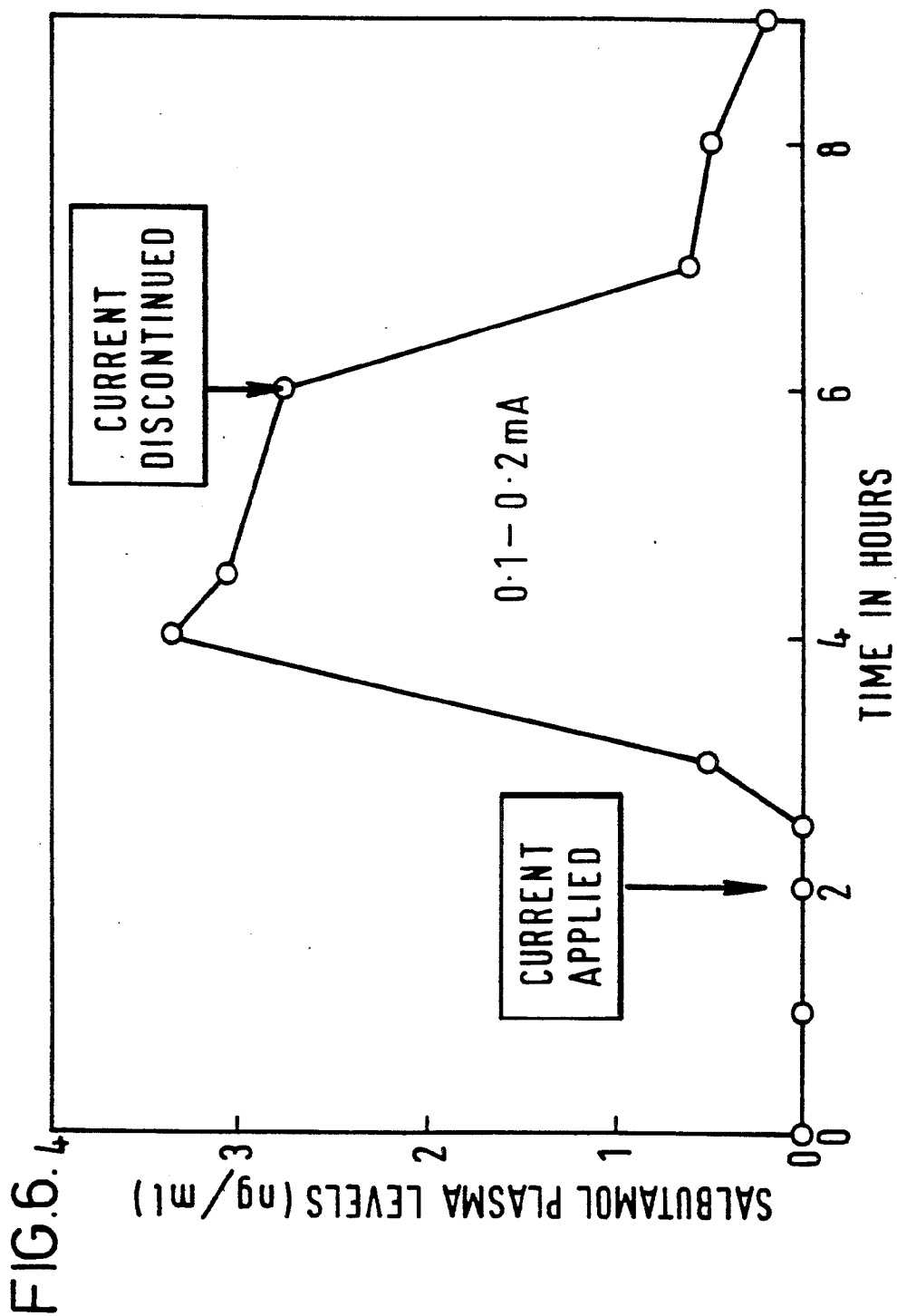
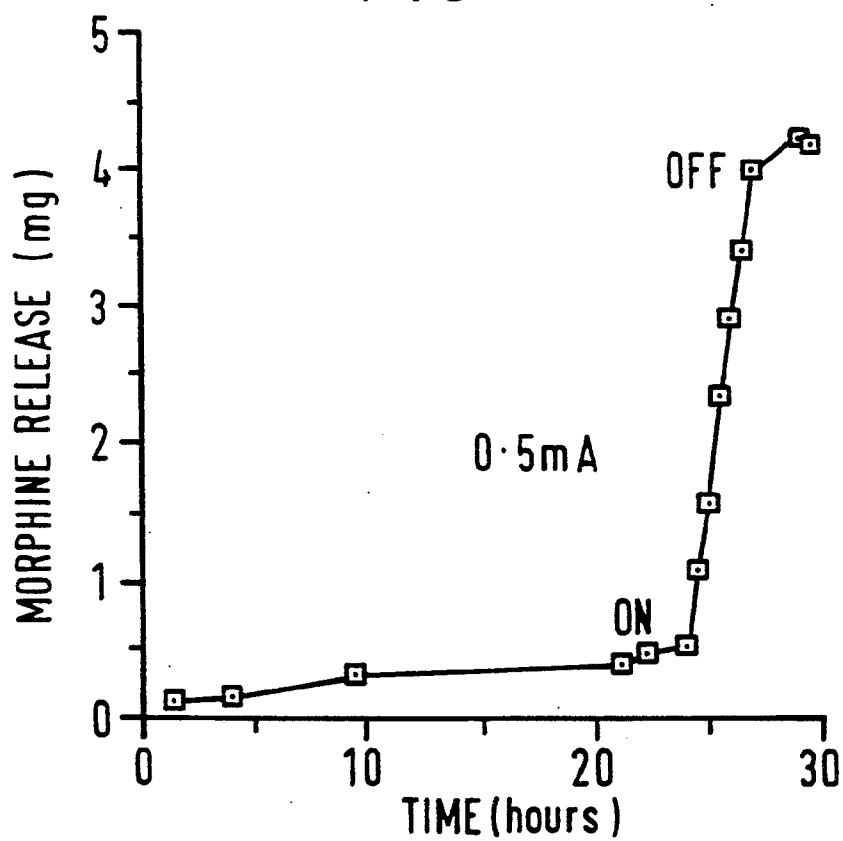


FIG. 7.



TRANSDERMAL DRUG DELIVERY DEVICE

This is a continuation of copending application Ser. No. 07/071,755 filed on Jul. 9, 1987 now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a device for the transdermal delivery of drugs and, in particular, to an electrode for use in a transdermal device which includes means for assisting or enhancing and controlling drug transport to the systemic circulation.

DESCRIPTION OF THE PRIOR ART

The study of the penetration of drugs through the skin has become increasingly important in recent years. The aims of such enhanced and controlled delivery are to maximize the bioavailability of the drug, to optimize the therapeutic efficacy and to minimize side effects.

There are many potential advantages of the transdermal route over the more conventional methods of drug administration. These advantages may be summarized in the following way.

Transdermal administration means that the drug may be introduced into the systemic circulation without initially entering the portal circulation where it may be metabolized into a pharmacologically inactive form (first pass effect). For drugs that are normally taken orally, administration through the skin can eliminate factors such as pH changes and food intake that influence gastrointestinal absorption. One of the most important advantages of the transdermal route is that it provides constant and continuous absorption of the drug, thus keeping blood levels within the "therapeutic window". In contrast, oral administration is often associated with variable absorption with blood levels sometimes rising to toxic levels or falling to subtherapeutic levels. The transdermal route is, therefore, a suitable route for the administration of very potent drugs, drugs with short half lives and low therapeutic indices or drugs which are subject to significant first pass effects.

Transdermal administration may allow rapid termination of drug input should side effects occur, and it increases patient compliance. The route is, however, clearly not suitable for drugs that seriously irritate or sensitize the skin, and for passive administration is restricted to drugs of suitable molecular configuration.

Many of the drugs that are otherwise suitable for transdermal delivery do not achieve sufficiently high blood levels for pharmacological activity when administered transdermally so that it is sometimes necessary to enhance this delivery. This can be achieved by chemical means namely by the use of absorption promoters e.g. aprotic solvents such as dimethylsulfoxide (DMSO), Azone (Trade Mark) and surfactants (Astley and Levine (1976) *J. Pharm. Sci.* 65, 210-215; Stoughton and McClure *Drug Dev. Ind. Pharm.* (1983) 9, 725-744).

In order that a transdermal delivery device may control the rate of penetration of the drug through the skin, it must release the drug at a rate which is less than that at which it can permeate the skin. Under these conditions, the more readily the drug is released from the drug delivery system, the higher the rate of transdermal absorption. The rate of drug release depends on whether the drug molecules are suspended or dissolved in the vehicle and on the interfacial partition coefficient of the drug between the delivery system and the skin.

A number of transdermal drug delivery systems have been developed and are currently in use. The drugs incorporated into these systems include nitroglycerin, which has been used for the treatment and prevention of angina pectoris, scopolamine for the treatment of motion sickness, the antihypertensive, clonidine and steroid hormones such as estradiol. These devices typically contain the active constituent dispersed or suspended in a reservoir: its rate of release is controlled either by matrix diffusion or by its passage through a controlling membrane.

The release characteristics of a number of these commercially available passive systems have been investigated by many researchers, including Chien, Y. W. (1983) *J. Pharm. Sci.* 72, 968-70, Dasta, J. F. and Gerates, D. R. (1982) and Shaw, J. E., et al (1976) *J. Invest. Dermatol.* 67, 677-678. Many other drugs are at present being evaluated for their suitability for transdermal administration.

The skin consists of three distinct layers; the epidermis, the dermis and subcutaneous fat. The outermost layer of the epidermis, the stratum corneum, is generally accepted to be the rate limiting barrier to drug penetration.

Hydration is one of the most important factors in skin penetration and may increase the absorption of substances that penetrate the skin Behl, C. R. et al (1983) *J. Pharm. Sci.*, 72, 79-82. Hydration results from water used in the preparation of the transdermal device.

The mobility of water molecules per se within the hydrated stratum corneum is crucial to the permeability of water soluble substances because they are very probably dissolved within this absorbed water. Just as diffusion in dilute aqueous solution requires cooperative motion of water molecules, the permeability of water-soluble substances through the stratum corneum likewise, depends on the mobility of water molecules surrounding the solute, Idson, B. J. (1975) *Pharm. Sci.*, 64, 901-924.

The rate of percutaneous absorption can be affected by the oil/water partition coefficient, the polarity of the drug and its degree of ionization, its solubility characteristics, molecular weight, volatility, concentration and the nature of the drug vehicle.

Many compounds evaluated for their ability to undergo percutaneous absorption are strong to weak electrolytes. Depending on the pK_a of the drug and the pH of the vehicle, such compounds exist in an equilibrium mixture of ionized and unionized species. To properly control the rate at which such electrolytes permeate the skin, it is necessary to determine the permeability coefficients of both forms of the drug. Michaels, A. S. et al (1975) *A. I. Ch.E.* 21, 985-996, calculated the permeabilities of the ionized forms of scopolamine and ephedrine to be 1/20th of those for the unionized forms. The permeation of the ionized drug through the skin is therefore possible and cannot be assumed to be negligible especially at pH levels at which large concentrations of ionized molecules are present, i.e., substances with low pK_a values, Swarbrick, J. et al (1984) *J. Pharm. Sci.*, 73, 1352-1355.

Other factors that affect the rate of absorption of drugs across the skin include chemical effects such as binding of the drug in the epidermis, (Zatz, J. L. (1983) *Drug Dev. Ind. Pharm.*, 9, 561-577 and the metabolism of the drug as it penetrates the skin, (Hadgraft, J. (1980) *Int. J. Pharm.*, 4, 229-239, Guy, R. H. and Hadgraft J. (1982) *Int. J. Pharm.*, 11, 187-197. The rate of percutan-

ous absorption is influenced by the temperature and increases as the temperature is raised. An increase in temperature may be effected by occluding the absorption site or by application of an absorption enhancer such as DMSO or a surfactant.

Anatomical differences in penetration rates seem to depend largely on the thickness of the stratum corneum, with rates increasing in the following anatomical order: plantar; anterior forearm; instep; scalp; scrotum; and posterior auricular.

The technique of iontophoresis has been used on a limited scale in medical therapy. Iontophoresis is the process of moving ions into surface tissues with the aid of an electrical current, Boone, D. C. (1982) in "Clinics in Physical Therapy: Electrotherapy", Ed. Wolf, S. L., Ch. 5, p 99-121. The technique was discovered nearly a century ago, but it is only in recent years that much interest has been shown in it as a method of local drug administration of ions; its chief proponents are to be found in the disciplines of dermatology, dentistry and otolaryngology. It is a safe, well documented method of introducing ions or polar substances into the skin by the application of a direct current between two electrodes placed on the skin of the patient e.g. pilocarpine, local anaesthetics, anti-virals (Gibson, L. W. and Cooke R. E. (1959) *Pediatrics*, 23, 545-549; Bridger M. W. M. et al, (1982) *J. Med. Eng. Tech.*, 6, 62-64; Ramsden R. T. (1977) *J. Laryngology and Otolology*, 91, 779-785; Johnson, C. and Shuster, S. (1970) *British J. Dermatol.*, 83, 367-379; Siddiqui, O. et al (1985) *J. Pharm. Pharmacol.*, 37, 732-735. One advantage claimed for iontophoresis as a technique for drug administration is that systemic toxicity is virtually eliminated, since only a small amount of drug is delivered. (Gangarosa L. P. et al (1978) *J. Pharm. Sci.*, 67, 1439-1443).

Transdermal devices are known from Patent Publication GB 2 104 388A and also from U.S. Pat. Nos. 4,557,723, 4,622,031 and 4,640,689. However, all of the devices disclosed in the aforementioned four documents are applied to the skin by adhesive means. The use of adhesive at the site of application of a drug which is to be administered by the transdermal route can cause severe irritation which may necessitate discontinuing such transdermal treatment. The irritation observed is frequently far more severe than the irritation caused by the drug itself which is sometimes observed at the site of application of a transdermal device.

It is an object of the present invention to provide an electrode for use in a transdermal device wherein the transport of drugs from a reservoir for the drug, integral with the electrode, to the skin and thence to the systemic circulation is promoted and controlled by means integral with the device and capable of supplying an electric current as a driving force for said drug transport.

It is a further object of the invention to provide a transdermal device which is portable and easy to operate and which can be readily adapted to meet the special requirements particular to a given drug.

SUMMARY OF THE INVENTION

Accordingly, the invention provides an electrode for use in a transdermal device, said electrode comprising a first surface adapted for contact with human skin and through which a drug substance contained in the electrode may pass to the skin under the influence of an iontophoretic or electro-osmotic force and a second surface remote from said skin-contacting surface which

is electrically conducting and which is adapted for contact with an electrical source in said transdermal device, said electrode having a surface area in contact with the skin, in use, which is in the range 0.1-30 cm², said drug being dissolved or dispersed in a hydrophilic medium in said electrode and said drug concentration being in the range 0.1 to 15% (w/v) based on the hydrophilic medium, and said second surface of said electrode being drug impermeable.

DETAILED DESCRIPTION OF THE INVENTION

Preferably, the hydrophilic medium is a gel material which is formed into a disc, one major surface of said disc defining said skin-contacting surface of said electrode and said other major surface of said disc having an electrically conducting material adhered thereto and defining said second surface of said electrode.

The disc of hydrophilic gel material may have a drug permeable membrane attached to said one major surface and defining said skin-contacting surface of said electrode and a layer of aluminium or platinum foil attached to said other major surface and defining said second surface of said electrode.

Preferably, the hydrophilic medium is a biocompatible polymer or polymeric gel of suitable rigidity and conductance and having the drug distributed throughout. A wide range of natural and/or synthetic polymeric materials or gelling agents or mixtures thereof may be used to form the hydrophilic medium of the transdermal device according to the invention. Such materials include agar gel, karaya gum gel, polyoxyethylene-polyoxypropylenes such as Pluronic F68 (Pluronic F68 is a Trade Mark) and Pluronic F127 (Pluronic F127 is a Trade Mark), gelatin, sodium carboxymethylcellulose, poly(ethylene oxide) polymers such as Macrogol (Macrogol is a Trade Mark), methylcellulose, carboxyvinyl polymers crosslinked with allyl sucrose such as Carbopol (Carbopol is a Trade Mark) and polyacrylamide gels or mixtures thereof. The term "agar" is synonymous with "agar-agar". The gelling agents may be based on aqueous solvents and co-solvents. The co-solvents include, for example, alcohols such as ethanol, polyols such as glycerol, ethylene glycol and propylene glycol, dimethylformamide, dimethylsulfoxide and other aqueous miscible co-solvents. The reservoir may also include suitable antimicrobial, antifungal and other pharmaceutical excipients *secundum artem*.

Suitable antimicrobial and antifungal agents/preservatives include benzalkonium chloride, cetrimide (cetyltrimethylammonium bromide), benzoic acid, benzyl alcohol, Parabens (Trade Mark for the methyl-, ethyl-, propyl- and butyl- esters of para-hydroxybenzoic acid), chlorhexidine, chlorobutanol, phenylmercuric acetate, borate and nitrate, potassium sorbate, sodium benzoate, sorbic acid and thiomersal (mercurithiosalicylate) or a mixture thereof.

The hydrophilic medium may also include an anti-oxidant. Preferred anti-oxidants include sodium metabisulphite, butylated hydroxyanisole and butylated hydroxytoluene or a mixture thereof.

The hydrophilic medium may also include a pH-controlling agent. Preferred pH-controlling agents include citric acid and sodium citrate.

The hydrophilic medium may also include a plasticizer. Suitable plasticizers include diethylphthalate, dibutylphthalate and tributylcitrate or a mixture thereof.

The hydrophilic medium may also include a surfactant. Suitable surfactants include sodium lauryl sulphate, diethylene glycol monostearate, propylene glycol monostearate, polyethylene glycols as sold under the Trade Mark MACROGOL, polysorbates and polyvinyl alcohol or a mixture thereof.

The hydrophilic medium may also include a penetration enhancer. Suitable penetration enhancers include dimethylsulfoxide, N,N-dimethylacetamide, N,N-dimethylformamide, 2-pyrrolidone, N-methyl-2-pyrrolidone and 1-dodecyl azacyclo-heptan-2-one or a mixture thereof.

The hydrophilic medium may also include a humectant. A particularly preferred humectant is glycerol for use in a high humidity environment.

Further the hydrophilic medium may also include a local anaesthetic. Suitable local anaesthetics include lidocaine, benzocaine, lignocaine, methocaine, butylaminobenzoate and procaine or a mixture thereof. The preparation would include a local anaesthetic mainly to suppress irritation at the site of application thereof caused by the drug.

Additionally, the hydrophilic medium may include a rubefacient. Particularly preferred rubefacients include camphor and menthol or a mixture thereof and other locally acting peripheral vasodilators.

The electrode according to the invention will normally have a contact area less than 10 cm².

In addition to the electrical source the essential components of the electrical circuit including the electrode hereinafter referred to also as said first electrode, are a means of adjusting the current, a means of indicating the successful operation of the device, i.e. an indicator light to show that the current is in the required range for the correct administration of the particular drug and a second electrode, which may be a counter electrode, which in use will be situated at a different site on the skin to said first electrode. The counter electrode will comprise a suitable metal or polymer such as a conductive resin or rubber and may contain a suitable conducting gel and/or an adhesive. The second electrode may also comprise an electrode of the type defined for said first electrode. Accordingly, the device incorporating the electrode according to the invention may be used to administer two drugs simultaneously by the transdermal route. When it is desired to administer two drugs of opposite charge, the first and second electrodes must be housed in chambers of opposite polarity.

More especially, the first electrode and the electrical source will be housed in a single unit which may also preferably include an LCD (liquid crystal display) and a control circuit. The LCD may display current, voltage and timing readings, as required. The exterior surface of the unit will, therefore, simulate the face of a time piece. The unit may include an ammeter and preferably a voltage adjuster. The control circuit may also include a galvanostat which regulates the current and maintains the current constant despite varying resistance of the skin. The power supply will suitably comprise conventional miniature or "light-weight" batteries. For example, conventional sheet batteries and microbatteries may be used.

The unit may also include a timing circuit which will activate the device at selected intervals or give a signal in the form of a bleep which will prompt the user to activate the device at selected intervals of time. However, the device can also be used for continuous admin-

istration of a drug and for continuous assisted drug transport.

The current used can be in the region of 0.01-10 mA. For example, the device can suitably operate at 0.5 mA at 10-20 volts. The current may be constant, variable or pulsed.

In a particularly preferred embodiment, the transdermal device includes a support means for attaching the device to a limb or appendage of the body. Such a support means is suitably in the form of a strap or bracelet, more particularly a wrist watch strap or bracelet. In place of a strap one may use a hollow bracelet. When a hollow bracelet is used the lead from the power supply to the counter electrode would be housed in the interior of the bracelet.

The second or counter electrode may be located in the bracelet or strap at a point distant from the first electrode or, alternatively, the two electrodes may be located adjacent to one another but separated by an insulating material.

The term "drug" as used herein embraces most pharmacologically active substances and also nutritional supplements such as vitamins and electrolytes. Especially suitable pharmacologically active substances for use as the drug in the electrode according to the invention include, for example, clonidine or a salt thereof, insulin, morphine, nicotine, orcipreniline or a salt thereof, salbutamol or a salt thereof, sodium chromoglycate and the peptide desmopressin. It will be appreciated that many drugs are actually administered in the form of a pharmaceutically acceptable salt.

As indicated above the device incorporating the electrode according to the invention may be used to administer two drugs simultaneously by the transdermal route. An example of drugs which may be suitably administered in this way are a combination of orcipreniline sulphate or salbutamol and sodium chromoglycate in the treatment of asthma.

Suitable concentrations for the preferred drugs for use in the electrode according to the invention are:

- nicotine 0.2-5% (w/v) based on the hydrophilic medium;
- clonidine 2-8% (w/v) based on the hydrophilic medium;
- salbutamol 1-6% (w/v) based on the hydrophilic medium;
- morphine 0.4-8% (w/v) based on the hydrophilic medium;
- orcipreniline 0.1-20% (w/v) based on the hydrophilic medium;
- sodium chromoglycate 1-10% (w/v) based on the hydrophilic medium;
- desmopressin 0.1-5% (w/v) based on the hydrophilic medium; and
- insulin 0.1-1% (w/v) based on the hydrophilic medium.

A particular advantage of the present transdermal device is that the electrode incorporating the drug reservoir defined by said hydrophilic medium forms an integral unit which can be discarded once the drug supply is used up. Hence one does not experience the problem which is characteristic of certain conventional transdermal devices which are used in association with an electrode and wherein the drug reservoir only is disposable. With such devices residues of material defining the drug reservoir adhere to the electrode when the drug supply of the reservoir is exhausted. Such residues build up with time, such that the device becomes pro-

gressively less effective and it becomes increasingly difficult to transport the drug to the skin surface in use.

The hydrophilic gel medium used in the electrode according to the invention is biocompatible, stable, easy to handle and compatible with the conducting material of the electrode.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be understood from the following description of an embodiment thereof given by way of example only with reference to the accompanying drawings in which:

FIG. 1a is a schematic representation of a transdermal device incorporating an electrode according to the invention;

FIG. 1b is a schematic representation of the electrode according to the invention;

FIG. 2 is a circuit diagram of the circuit employed in the transdermal device depicted in FIG. 1a;

FIG. 3 is a plot of in vitro nicotine release (mg) versus time (hours) for the electrode as prepared in Example 1;

FIG. 4 is a plot of in vitro clonidine release (mg) versus time (hours) for the electrode as prepared in Example 2;

FIG. 5 is a plot of in vitro salbutamol release (mg) versus time (hours) at 0.25 mA (curve a) and 0.5 mA (curve b) for the electrode as prepared in Example 3;

FIG. 6 is a plot of in vivo salbutamol plasma levels (ng/ml) versus time (hours) for the electrode as prepared in Example 3; and

FIG. 7 is a plot of in vitro morphine release (mg) versus time (hours) for the electrode as prepared in Example 4.

Referring to FIG. 1a of the drawings, there is indicated generally at 1 a transdermal device incorporating a disposable electrode 3 according to the invention, said device 1 comprising a housing 2 for the electrode 3 (FIG. 1b) which consists of an electrically conducting layer 4 and a disc of 4% agar gel 5 in which is dispersed salbutamol at a concentration of 27.5 mg/ml and which is attached to the site of application by means of a strap 6 having at the free ends thereof the cooperating elements of a conventional clasp 7. The electrode 3 is connected by a lead 8 to a source of electrical potential comprising a power supply 9, a control and timing circuit 10, an ammeter 11, a galvanostat 12 and a fixed metal electrode 13 against which is placed the conducting layer 4 of the electrode 3.

The power supply 9 is also connected via a lead 14 to a counter electrode 15 located adjacent the clasp 7 and which electrode 15, in use, allows the circuit to be completed when the device is applied to the skin. The electrode 15 comprises a layer of a conducting gel, one major surface thereof defining a skin-contacting surface and the other major surface thereof having intimately associated thereto a metallic conducting layer. The power supply 9 comprises two miniature batteries (2.5 V). The device 1 also contains an LCD 16 with appropriate switching arrangements which can display time, current and voltage, an audible alarm/warning device which prompts the user to activate the device by pressing an on/off button 17, and an LED (light emitting diode) to indicate satisfactory operation of the device.

The main components of the circuit employed in the device 1 are depicted in the circuit diagram comprising FIG. 2. Said components are as follows:

TC — a timing circuit, optionally programmable and with an audible warning device;

PS — a power supply with reversible polarity;

A — an ammeter;

G — a galvanostat;

SS — a selective switch;

LCD — a liquid crystal display for time, voltage or current, as selected;

LED — a visible signal of satisfactory operation of the device.

The invention will be further illustrated by the following Examples.

EXAMPLE 1

A nicotine-containing agar gel was prepared by dispersing 4% agar in glycerol:water (1:4) and dissolving nicotine (98-100% anhyd.; Sigma Chemicals N3876) therein so as to achieve a concentration of 55 mg/ml. While still in the liquid state, the gel so prepared was spread on a layer of aluminium foil so as to obtain an electrode according to the invention having a surface area of 8 cm².

In vitro release of nicotine from the electrode so prepared was determined in a glass, custom built diffusion cell based on the Franz cell (Franz T. J. (1975) J. Invest. Dermatol., 64, 190). Full thickness abdominal skin (approx. 4 cm × 15 cm) taken from cadavers within 48 hours post mortem was used in the in vitro characterisation as the transport membrane. The stratum corneum and epidermis (SCE) were separated from the other skin layers using the method of A. M. Kligman and E. Christophers (Archives Dermatology (1963) Vol. 88, pages 702-705). The nicotine transported through the membrane was analysed using a Pye Unicam SP 200 (Trade Mark) uv/vis spectrophotometer and by reverse phase HPLC. The nicotine release is depicted in FIG. 3 of the accompanying drawings.

EXAMPLE 2

Example 1 was repeated except that nicotine was replaced by clonidine hydrochloride and the clonidine hydrochloride was dissolved in 4% agar gel so as to achieve a concentration of clonidine hydrochloride of 27 mg/ml. The in vitro release of clonidine was measured according to the procedure of Example 1 and the release is depicted in FIG. 4 of the accompanying drawings.

EXAMPLE 3

Example 1 was repeated except that nicotine was replaced by salbutamol and the salbutamol was dissolved in a gel made from methylcellulose (0.16%) and agar (3.84%) so as to achieve a concentration of salbutamol of 27.5 mg/ml. The in vitro release was measured according to the procedure of Example 1 and the release is depicted in FIG. 5 of the accompanying drawings.

The release of salbutamol from the electrode so prepared was also measured in vivo in two subjects and the mean results are depicted in FIG. 6 of the accompanying drawings.

EXAMPLE 4

Example 1 was repeated except that nicotine was replaced by morphine and the morphine was dissolved in 5% agar gel so as to achieve a concentration of 55 mg/ml. The in vitro release was measured according to the procedure of Example 1 and the release is depicted in FIG. 7 of the accompanying drawings.

EXAMPLE 5

Example 1 was repeated except that nicotine replaced by desmopressin and the desmopressin was dissolved in a gel made from karaya gum (30%) so as to achieve a concentration of desmopressin of 3 mg/ml.

EXAMPLE 6

Example 1 was repeated except that nicotine was replaced by insulin and the insulin was solubilized in a 30% aqueous gel containing polyacrylamide (approx. 15×10^6 molecular weight) so as to obtain a concentration of insulin of 4 mg/ml.

EXAMPLE 7

Separate electrodes containing sodium chromoglycate and salbutamol, respectively, were prepared for use in a transdermal device for the simultaneous administration of said drugs by the transdermal route. The sodium chromoglycate electrode was prepared according to the procedure of Example 1 except that nicotine was replaced by sodium chromoglycate and the sodium chromoglycate was dissolved in 4% agar gel so as to achieve a concentration of 30 mg/ml. The salbutamol electrode was prepared in accordance with Example 3 and contained a concentration of salbutamol of 27.5 mg/ml.

What we claim is:

1. A transdermal device suitable for long term administration of a drug substance comprising: a housing; a complete electrical circuit having a first electrode detachably mounted within a recess in said housing, said first electrode having a first surface adapted for contact with human skin and through which the drug substance contained in the first electrode may pass to the skin under the influence of an iontophoretic or electroosmotic force and a second surface remote from said skin-contacting surface which is electrically conducting and which is adapted for contact with an electrical source in said housing such that the first electrode can be discarded and replaced by a new electrode when the drug supply substance is exhausted, said first electrode having a surface area adapted to be in contact with the skin when in use, said drug substance being dissolved or dispersed in a hydrophilic medium defining said first electrode, and said second surface of said first electrode is drug impermeable, a means for adjusting the current, means for indicating the current is in the required range for correct administration, and a second electrode which is optionally detachable for completing said electrical circuit; and a non-adhesive means for securing said device to the skin.

2. A transdermal device according to claim 1, wherein the hydrophilic medium is a gel material which is formed into a disc, one major surface of said disc defining said skin-contacting surface of said first electrode and a second major surface of said disc having an electrically conducting material adhered thereto and defining said second surface of said first electrode.

3. A transdermal device according to claim 2, wherein the disc of hydrophilic gel material has a drug permeable membrane attached to said one major surface and defining said skin-contacting surface of said first electrode and a layer of aluminium or platinum foil attached to said second major surface and defining said second surface of said first electrode.

4. A transdermal device according to claim 3, wherein the hydrophilic medium is selected from the

group consisting of agar gel, karaya gum gel, a polyoxyethylene-polyoxypropylene gel, gelatin, sodium carboxymethylcellulose, a poly(ethylene oxide) polymer, methylcellulose, carboxyvinyl polymers cross linked with allyl sucrose and polyacrylamide gels or a mixture thereof.

5. A transdermal according to claim 1, wherein the hydrophilic medium contains one or more additional agents selected from the group consisting of antimicrobial agents, antifungal agents, preservatives, anti-oxidants, pH-controlling agents, plasticizers, surfactants, penetration enhancers, humectants, local anaesthetics and rubefacients.

6. A transdermal device according to claim 1, wherein the second electrode connected to said housing such that, when in use, will be situated at a different site on the skin to said first electrode.

7. A transdermal device according to claim 6, wherein the components of the electrical circuit, excluding the second electrode, are housed in a single unit, the exterior surface of said unit simulating the face of a time piece, said unit being mounted in a strap or bracelet for attachment to a limb of a body.

8. A transdermal device according to claim 7, wherein the unit includes an LCD and a galvanostat which regulates the current applied to the first electrode and maintains said current constant despite the varying resistance to the skin.

9. A transdermal device according to claim 1, wherein the drug substance is selected from the group consisting of clonidine, insulin, morphine, nicotine, oripreniline, salbutamol, sodium chromoglycate and desmopressin or a pharmaceutically acceptable salt thereof.

10. A transdermal device according to claim 1, wherein said second electrode is also an electrode of the type as defined for said first electrode, such that the transdermal device incorporating said first and second electrodes may be used to administer two drugs simultaneously by the transdermal route.

11. A transdermal device according to claim 1, wherein the surface area of the first electrode adapted to be in contact with the skin is in the range of 0.1 to 30 cm².

12. A transdermal device according to claim 1, wherein the drug substance has a concentration in the range of 0.1 to 15% weight/volume based on the hydrophilic medium.

13. A transdermal device comprising a housing first electrode detachably mounted to said housing and an electrical source within said housing wherein said housing is mounted in a strap or bracelet for attachment to a limb of a body, said first electrode having a first surface with a surface area adapted for contact with human skin when in use, a drug substance dissolved or dispersed in a hydrophilic medium defining said first electrode which can pass to the skin under the influence of an iontophoretic or electroosmotic force created by said electrical source, and a second surface remote from said first surface which is drug impermeable, electrically conducting and an integral part of said first electrode, said second surface further being adapted for contact with said electrical source, and a second electrode in said strap or bracelet for connection to said electrical source and adapted to be situated at a different site on the skin to said first electrode.

14. The transdermal device according to claim 13, wherein said second electrode is also an electrode of the

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type as defined for said first electrode, such that the transdermal device incorporating said first and second electrodes may be used to administer two drugs simultaneously by the transdermal route.

15. A transdermal device according to claim 13, wherein the surface area of the first electrode adapted

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to be in contact with the skin is in the range of 0.1 to 30 cm².

16. A transdermal device according to claim 13, wherein the drug substance has a concentration in the range of 0.1 to 15% weight/volume based on the hydrophilic medium.

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United States Patent [19]

Sibalis et al.

[11] **Patent Number:** **4,878,892**[45] **Date of Patent:** **Nov. 7, 1989**[54] **ELECTROLYTIC TRANSDERMAL
DELIVERY OF POLYPEPTIDES**[75] **Inventors:** Dan Sibalis, Stony Brook; Sanford
Rosen, New York, both of N.Y.[73] **Assignee:** Drug Delivery Systems Inc., New
York, N.Y.[21] **Appl. No.:** 12,889[22] **Filed:** Feb. 10, 1987[51] **Int. Cl.⁴** A61N 1/30[52] **U.S. Cl.** 604/20[58] **Field of Search** 604/20[56] **References Cited****U.S. PATENT DOCUMENTS**

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| 4,710,497 | 12/1987 | Heller et al. | 514/947 |
| 4,713,244 | 12/1987 | Baum et al. | 514/912 |
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Primary Examiner—C. Fred Rosenbaum*Assistant Examiner*—Mark O. Polutta*Attorney, Agent, or Firm*—Lackebach, Siegel Marzullo
& Aronson[57] **ABSTRACT**

The invention comprises in combination:

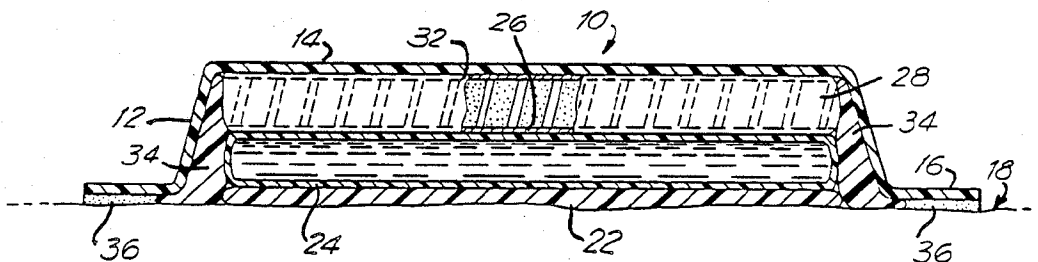
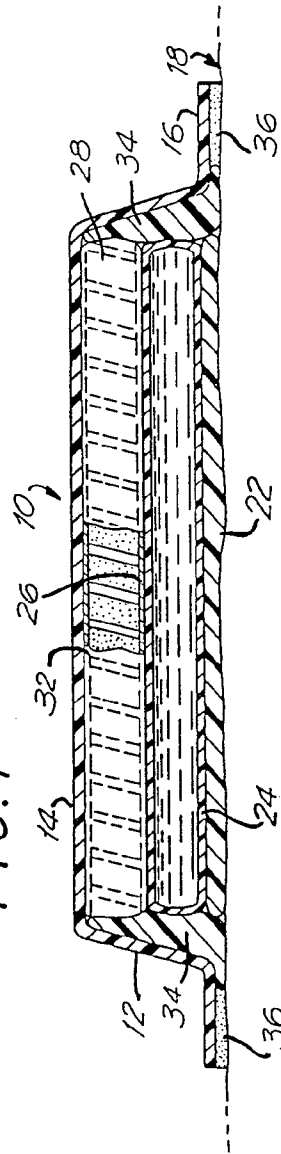
(a) a polypeptide having from about three to about 20
peptide units in aqueous solution or suspension, and(b) an electrolytic device for transdermal transport of
the polypeptide to the bloodstream of the patient.It may be useful to enhance the transdermal delivery of
the polypeptide by adding an aqueous cosolute/cosol-
vent with negative Setschenow constants.The electrolytic device preferably comprises a hydro-
philic reservoir containing a supply of the aqueous poly-
peptide solution or suspension, an electric battery, two
extended contacts, and optionally a semipermeable
membrane between the reservoir and the patient's skin.Representative polypeptides include oxytocin, angio-
tensin I, II, and III, substance P, vasopressin, lyppressin,
desmopressin, leuprolide acetate, antripectin, and the
like.**19 Claims, 1 Drawing Sheet**

FIG. 1



ELECTROLYTIC TRANSDERMAL DELIVERY OF POLYPEPTIDES

RELATED U.S. PATENTS AND APPLICATIONS

This application is related to U.S. Pat. Nos. 4,557,723, 4,622,031 and 4,640,689 and to copending applications Ser. No. PCT/US85/00080 filed Jan. 17, 1985; PCT/US85/01074 and PCT/US85/01075 both filed June 10, 1985; Ser. No. 778,183 filed Sept. 16, 1985; Ser. No. 807,234 filed Dec. 10, 1985; Ser. No. 839,523 filed Mar. 14, 1986; Ser. No. 888,151 filed July 18, 1986; Ser. No. 922,296 filed Oct. 23, 1986; and Ser. No. 554 and 555 filed Jan. 5, 1987.

FIELD OF THE INVENTION

This invention relates to electrolytic transdermal delivery of polypeptides and more specifically to delivery to the blood stream of the patient of aqueous solutions or suspensions containing polypeptides with from about three to about 20 alphaamino acid units.

BACKGROUND OF THE INVENTION

Patents and patent applications cited above disclose basic aspects of transdermal delivery of drugs by electrical power patches on the patient's skin. Other U.S. and foreign patents also disclose transdermal electrical, and medical effects, as follows:

| U.S. Pat. No. | | |
|-----------------|-----------------|--------------|
| 385,556 | 2,267,162 | 3,163,166 |
| 486,902 | 2,493,155 | 3,289,671 |
| 588,479 | 2,784,715 | 3,547,107 |
| 3,677,268 | 4,239,052 | 4,367,745 |
| 4,008,721 | 4,243,052 | 4,367,745 |
| 4,141,358 | 4,273,135 | 4,406,658 |
| 4,164,226 | 4,290,878 | 4,419,019 |
| 4,166,457 | 4,325,367 | 4,474,570 |
| 4,239,046 | 4,362,645 | |
| Foreign Patents | | |
| EPA 58,920 | DE 2,902,021.83 | UK 2,104,388 |
| EPA 60,452 | DE 3,225,748 | |

None of these references, however, show the effective administration of polypeptide drugs such as desmopressin, vasopressin, substance P, angiotensin, lypressin and the like.

OBJECTS OF THE INVENTION

It is an object of the present invention to administer polypeptide drugs with a range of molecular weights from about three peptide units to about 20 peptide units transdermally to humans, adult or child, and other animal patients by means of a locally applied electric field.

It is a further object of the invention to administer polypeptide drugs transdermally in an electric field regardless of the degree of ionization or the amount of ionic charge on the polypeptide.

It is yet another object to maximize the transdermal administration of polypeptide drugs by eliminating or minimizing the association of polypeptide drugs in aqueous media.

It is still another object to administer polypeptide drugs transdermally by an electric applicator which

occupies minimal area, gives the patient minimal discomfort, generates sufficient current density with minimal size and weight, and operates effectively under a wide variety of skin conditions.

It is yet a further object to administer polypeptide drugs transdermally by electrolytic devices without irritation or reddening of the skin, and without tingling or other sensations.

Other objects of the present invention will be apparent to those skilled in the art.

SUMMARY OF THE INVENTION

The present invention comprises in combination:

- (a) a polypeptide having from about three to about 20 peptide units in aqueous solution or suspension, and
- (b) an electrolytic device for transdermal transport of the polypeptide to the bloodstream of the patient.

The invention further comprises a method for delivering the polypeptide to the bloodstream of the patient by means of the electrolytic device.

The polypeptide may be of homopolymeric, heteropolymeric, cyclical, or other structural type.

It may be useful to enhance the transdermal delivery of the polypeptide by adding an aqueous cosolute/-cosolvent with negative Setschenow constants.

The electrolytic device preferably comprises a hydrophilic reservoir containing a supply of the aqueous polypeptide solution or suspension, an electric battery, two extended contacts, and optionally a semipermeable membrane between the reservoir and the patient's skin.

Representative polypeptides include oxytocin, angiotensin I, II, and III, substance P, vasopressin, lypressin, desmopressin, leuprolide acetate, and the like. Within the scope of this invention is the transdermal delivery of polypeptides with other classes of drugs, such as steroids.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a cross-sectional view of one type of electronic/electrolytic device to administer polypeptides to a patient transdermally.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Polypeptides are condensation polymers of aminoacids which are linked by formation of amide bonds from the amino group of one aminoacid and the carboxylic acid group of another. Polypeptides are homopolymers or heteropolymers of the 20 aminoacids necessary to build mammalian body tissue. In nature, they have a laevo configuration. In this disclosure "polypeptide" means molecules with from about three to about 20 peptide units.

In copending application Ser. No. 012,898, filed currently on Feb. 10, 1987 I disclose an invention for administering "proteins" transdermally by electrolytic means and define "protein" as a polypeptide with more than about 20 peptide units, e.g. insulin whose single-strand has 51 units.

Numerous polypeptides are useful drugs in the treatment of human diseases or their diagnosis. Table I shows examples of the names of drugs, their trade names, and their application.

TABLE I

| Some Polypeptide Drugs | | | |
|------------------------------------|---------------|----------------------|--|
| Name | Tradename(s) | No. of Peptide Units | Application |
| vasopressin | Pitressin | 9 | diabetes insipidus GI hemorrhage |
| desmopressin | DDAVP | 9 | diabetes insipidus |
| oxytocin | Pitocin | 9 | induction of labor |
| lypressin | Diapid | 9 | antidiuretic |
| leuprolide acetate | Lupron | 9 | prostate cancer LH-RH FSH inhibitor |
| dynorphin A (1-17) | | 17 | peptide opioids |
| dynorphin A (1-8) | | 8 | |
| dynorphin B | | 13 | |
| met-enkephalin | | 5 | are analgesics |
| leu-enkephalin | | 5 | |
| thyrotropin releasing hormone | TRH | 3 | clinical diagnostic |
| | MIF | 3 | |
| α -melanocyte stim. hormone | α -MSH | 13 | hormonal regulator |
| β -melanocyte stim. hormone | β -MSH | 18 | hormonal regulator |
| neurotensin | | 13 | insulin regulator |
| substance P | | 11 | analgesic |
| somatostatin | | 14 | growth hormone inhibitor |
| angiotensin I | | 10 | modulate blood pressure |
| II | | 8 | |
| III | | 7 | |
| atriopeptin | | 21 | fluid regulation |

It should be emphasized that polypeptides can vary widely in the number of peptide units they contain from a few, as in thyrotropin releasing hormone to six times 50 51 units as in hexameric insulin. In order for large polypeptides to be transported through the skin, it is preferable that the polypeptide is unassociated. If the polypeptide is single-stranded, the cross-section of the linear macromolecular would be only a few square Angstroms even if the entire folded, convoluted, or random polymer is large in radius of gyration. It is sometimes preferable to add a dissociating agent to a solution of the smaller polypeptides of the present invention, also.

There have been many proposed descriptive models 40 for the structure of liquid water, such as:

- (a) in interstitial model of ice whose cavities are filled with water,
- (b) quartz-like aggregates
- (c) water as a hydrate of itself,
- (d) flickering clusters of cooperative H-bonds;
- (e) a two-structure, mixture model.

Chemists have known for decades that water forms clathrates with xenon, chlorine, methane and other molecules and therefore must have cavities. The structure of liquid water depends on the distance and angles of H-bonds. In a two-dimensional sense, water is a hexagonal array of "aromatic" structures.

The solubility of argon in "structured" water is about one-tenth that of argon in alcohols. As temperature increases from 0° to 30° C. the solubility of argon in water decreases while that in alcohols increases. The charge in entropy of methane in water is about -15 to -20 e.u. but in alcohols, dioxane, and cyclohexane is about -1 e.u. The change in enthalpy of methane in water is about -3000 cal/mole, but in the same organics is 200-500 cal/mole. The process of solution may be modeled as "forming a cavity" then introducing the solute into that cavity. In a normal fluid the energy to form the cavity is positive, then filling the cavity is minus (attractive). Since water already has cavities present, there is about zero energy to form such and negative energy to dissolve the solute (fill) the cavity.

Adding some nonpolar nonelectrolytes such as either, methyl acetate, dimethylsulfoxide structure water i.e. reinforce water structure and decrease its compressibility. Some small ions e.g. lithium and fluoride also reinforce the structure of water.

Conversely, most ions, iodine, methyl halides, small aminoacids, urea, and other polar nonelectrolytes are "structure breakers" of water.

Precise analysis of the structure of water may be a complex matter, yet it is a description of only one substance. Precise description of polypeptides dissolving in water, or dissociating if already dissolved, covers much broader phenomena, since there are myriad polypeptides and myriad cosolvents or dissociation agents to coact with water.

The conformation of polypeptides in solution is dependent at a minimum on the concentration of polypeptide, pH, solvent composition, ionic strength, ionic charge on the polypeptide, solvent dielectric properties, presence of cosolutes, shear stresses, and the presence of heterogeneous third bodies such as surfaces of the container, granules, and the like.

It is generally accepted that the configuration of polypeptides in aqueous media comprises folded macromolecules with hydrophobic domains forming a central core and hydrophilic domains oriented toward the aqueous perimeter. The process of dissolution is difficult to describe in detail, but the energetics of the solution process can be determined in a straightforward manner. Much information about solution, disassociation, denaturation, coiling, gelation, unfolding, and other changes in so-called tertiary and quaternary structures may be gained from a detailed study of solution and/or gelation of polypeptides in water and water containing other cosolvents or "agents".

The primary structure of a polypeptide is the term used for the sequence of aminoacids as they appear along the chain of the macromolecule. The local organization of the chain e.g. helix formation, random coil, folding is termed secondary structure. The overall spatial arrangement of the polypeptide on the atomic level,

what X-ray crystallography shows, is the tertiary level of structure. The quaternary structure is that of several chains which may form different regions with different properties e.g. a dumbbell-like structure with a flexible middle rod and two hard ends. The function of the regions may vary. In hemoglobin, 4 myoglobins group to form a dumbbell shape with a molecular weight of about 17,000 daltons with the oxygen-bearing function associated with the two harder spheres on the ends rather than the flexible part in the middle.

Dissociation agents greatly affect quaternary structure, are irrelevant to tertiary structure, may affect secondary structure, and have no effect on primary structure of polypeptides.

The effect of a solvent such as water on a polypeptide can be described in terms of an equilibrium constant K_D and the standard free energy of dissociation ΔF° , when a polypeptide dissociates from e.g. hexamers to dimers or single-stranded subunits e.g. insulin in water. Often these different fragments can coexist in a series of equilibria e.g. earthworm hemoglobin duodecamers, hexamers, tetramers, dimers, and single fragments, at intermediate concentrations of a pure solvent or one with a dissociation agent such as propylurea or sodium perchlorate present. When such an added dissociation cosolvent is present there are two dissociation constants K_{DW} and K_{DAW} , where DW designates pure water and DAW designates added agent and water. The interaction of the added agent and the polypeptide involves the binding constant K_B .

For polypeptides binding constant K_B is the summation of two terms: a polar component K_P related to the peptide bond $-\text{NHCOO}-$ and a hydrophobic component K_H related to the average hydrophobic moiety $-\text{CHR}-$ different from each aminoacid but averageable. The constant is related to energetics by the Nernst equation. So

$$F^\circ_{DW} = F^\circ_{DAW} - mNRT(K_P + K_H)[\text{da}],$$

when m is the number of fragments and N is the number of binding sites and $[\text{da}]$ is the concentration of the dissociation cosolvent.

When a solid polypeptide is in contact with a well-stirred solvent such as water for a long time (e.g. a week) an equilibrium saturated solution is established:

$$K_{eq} = -RT \ln C_{sat}$$

When another compound is added to the water, such as an electrolyte or a nonelectrolyte, a different C_{sat} is established at equilibrium. This other value will normally be different from C_{sat} for pure water. The higher the concentration of the added agent, the higher (or lower) the saturated concentration of the polypeptide. When one graphs the $\log C_{sat}$ against the molarity of the added agent, a straight line is formed. The slope of this straight line is known as the Setschenow constant for the agent. Since the equation above has a minus sign in it, those agents which aid solubility and dissociation, e.g. urea, have a negative Setschenow constant, and those agents which decrease solubility and dissociation, e.g. sodium or ammonium sulfate have positive Setschenow constants.

$$K_s \approx -K_B/2.303$$

The Setschenow constant K has peptide and hydrophobic components. The Setschenow constant can be approxi-

mated by negative K_B divided by log transform constant 2.303. Since negative standard free energies of transfer indicate spontaneous reactions, negative F° values for transfer from water to a mixture of water and the cosolvent indicate dissociation. The more negative, the more dissociated. Table II gives Setschenow constants for average peptide and hydrophobic groups as well as free energy of transfer values for a variety of cosolvents, as taken from a paper by Herkovits et al., Journal of Colloid and Interface Science, vol. 63, No. 2, p. 232 of February 1978. The lower the position in Table II, the better the dissociation agent.

Since thermodynamics is a description of the ultimate reality, the last column listing free energies of transfer shows those agents which are preferred in practicing the present invention, those agents with negative standard free energies. The Setschenow constants are helpful, however, in appreciating how the agent is useful. The "sum" column of interaction with peptide linkages in the polypeptide plus the interaction with the hydrophobic moieties is directly linked to the free energy column by the Nernst equation. It is the peptide interaction number and the hydrophobic or "methylene" number, which show how a dissociation agent works.

TABLE II

| Agent | Setschenow Constants | | | -F. [°] cal/mole |
|-------------------------|----------------------|---------------------------|--------|------------------------------|
| | For peptide | For -CH ₂ - | Sum | |
| Sodium sulfate | -0.013 | 0.085 | 0.072 | 98 |
| Potassium fluoride | -0.027 | 0.05 | 0.023 | 31 |
| Ethanol | +0.037 | -0.014 | 0.023 | 31 |
| Dioxane | +0.029 | -0.013 | 0.016 | 22 |
| Sodium chloride | -0.037 | 0.033 | -0.004 | -5 |
| Sodium acetate | — | — | -0.009 | -12 |
| Sodium bromide | -0.037 | 0.025 | -0.012 | -16 |
| Calcium chloride | -0.077 | 0.063 | -0.014 | -19 |
| Sodium propionate | — | — | -0.017 | -23 |
| Urea | -0.018 | -0.01 | -0.028 | -38 |
| Sodium butyrate | — | — | -0.038 | -51 |
| Propylurea | — | — | -0.047 | -64 |
| Sodium thiocyanate | -0.077 | 0.007 | -0.07 | -96 |
| Potassium iodide | -0.083 | 0.01 | -0.073 | -100 |
| Sodium perchlorate | -0.097 | 0.021 | -0.076 | -104 |
| Sodium iodide | -0.087 | 0.01 | -0.077 | -105 |
| Guanidine hydrochloride | -0.061 | -0.027 | -0.088 | -120 |

Urea, guanidine hydrochloride, or any other compound which has two negative parameters interact with the entire polypeptide to disaggregate any quaternary structure and perhaps unfold the secondary structure. This type of dissociating agent may be helpful in practicing the present invention of delivering polypeptides and from about three to about 20 peptide units to the bloodstream of the patient. Sodium perchlorate, potassium iodide, and the like interact so strongly with peptide bonds that their lack of interaction with hydrophobic linkages of the polypeptide does not appreciably inhibit dissociation of the polypeptide. These agents may be useful in practicing the present invention. Ethanol, dioxane and other organics strongly react with the hydrophobic moieties, but not enough to overcome the nonpolar nature of organic solvents. Data on ethanol diverges, however. Such agents have limited utility in practicing this invention. Agents which have two positive components for their Setschenow constant and hence a positive standard free energy of transfer do not appear on Table II.

Electrophoresis is the transport of both solute and solvent in an electric field. Ionophoresis is the transport of charged ions by coulombic attraction/repulsion in an electric field. Electroosmosis is the transport of solvent in an electric field.

Many workers in the prior art overemphasized ionophoresis and underestimated electroosmosis in their analysis of both the best modes for and problems associated with transdermal delivery of drugs by electrolytic means. In fact, the essence of transdermal, electric-powered delivery of drugs is that control and maximization is central regardless of whether the drug is transported by coulombic attraction/repulsion or electroosmotic solvent streaming. In the present invention, unlike the prior art, Faraday's law is irrelevant. In many situations, more drug is carried by electroosmosis than ionophoresis, so that the amount of charge or degree of ionization of the polypeptide is not important. Before the present invention this fact was not appreciated. Prior workers attempted to improve ionophoresis by increasing charge density on the polypeptide by oxidation or hydrolysis. For this invention the value of charge density on the drug does not control the dosage.

Electronic conduction is the movement of electrons in an electric field. Electrolytic conduction is the movement of ions in an electric field. Prior to the present invention, many workers failed to communicate their results well or to explain their ideas well because of confusion regarding the flow of electrons and the flow of ions. In the applicator of the present invention, current flow in the electrodes is electronic and current flow in the reservoir and through the skin is electrolytic, but is possible to have some electronic flow along the chain of a polypeptide in an electric field in water or aqueous media.

The values of the electrical variables in the practice of the current invention in vivo are those pertaining to electroosmosis not ionophoresis. The current density may range from about 0.5 $\mu\text{A}/\text{cm}^2$ to about 1 mA/cm^2 , preferably about 0.5 $\mu\text{A}/\text{cm}^2$ to about 10 $\mu\text{A}/\text{cm}^2$ rather than 1 mA/cm^2 to 5 mA/cm^2 values associated with ionophoresis. The voltage impressed for operating the applicator of the present invention ranges from about 1 to about 40 volts rather than the 50 to 100 or more volts advisable for ionophoresis. Likewise the migratory flow of water in an electrolytic field are the much higher values of about 0.001 $\text{ml}/\text{cm}^2/\text{hr}$ to about 0.005 $\text{ml}/\text{cm}^2/\text{hr}$ constant of electroosmosis not the typical adventitious values for ionophoresis, following Faraday's law which impels only ions.

It is highly preferred that the current density employed in the present invention be low enough to prevent any irritation, reddening, inflammation, or erythema in the skin of the patient. In addition to the polypeptide drug, there may be salts for physiological balance, buffering agents, biocides, preservatives, disinfectants, antibiotics, or other additives in the composition of the drug reservoir of the electrolytic transdermal device.

It is sometimes useful to add chelating agents to the drug. Some of the metal ions which may be associated with the polypeptide are magnesium, zinc, copper, chromium, cobalt, nickel, iron, and manganese. Many conventional chelating agents may be employed such as the salts of ethylenediaminetetraacetic acid (EDTA). Other conventional chelating agents may also be used.

FIG. 1 shows generally drug applicator 10 comprising outer cover 12 having a raised portion 14 and an outer-edge lip 16 in contact with the skin 18 of the patient. The layered structure of the drug applicator 10 can be any convenient and effective size or shape such as rectangle, oval, circle, or splayed shape to fit crevices at interfaces of body parts. The size of the applicator may range from about 10 cm^2 to about 200 cm^2 depending on its use and the species, age, and size of the patient.

Applicator 10 often has generally a structure of horizontal layers. The layer shown in FIG. 1 is that closest to the skin 18 is an optional semipermeable membrane 22 through which the drug diffuses for deposition on skin 18. Optional membrane 22 may be constructed of semipermeable cellulose acetate, poly(vinyl chloride), or regenerated cellulose.

Above optional semipermeable membrane 22 is a reservoir, region, or pouch 24 for holding the supply of the drug to be electrolytically delivered. Preferably reservoir 24 defines a closed space and is flexible. Typical materials used in forming pouch 24 are rayon fluff, polyurethane sponge, and hydrophilic adhesives. This reservoir may also consist of a hydrophilic gel. For containing the polypeptide solution or suspension of the present invention, reservoir 24 may range from about 0.01 ml to about 15 ml in volume, preferably about 0.15 ml to about 0.9 ml for about a week's continual administration of a polypeptide drug in amounts ranging from about 500 nanograms to 1 mg per day, depending on the size, species, and age of the patient. The gel, pouch, or walls of the reservoir 24 must be microporous enough to transfer of the solvent, solution, or suspension of the polypeptide by the electric field, but not so porous to allow leakage of the suspension or solution of the polypeptide drug. The choice of whether or not to employ optional semipermeable membrane 22 is interrelated with the choice of design and material of reservoir 24, because their functions may overlap.

The next higher layer above reservoir 24 is shown in FIG. 1 comprises extended contact 26 which is preferably the lower face of battery 28. Contact 26 preferably is flexible enough to conform to the surface of the skin and also is electronically conductive. Preferred materials for contact 26 are electric-conducting polymers, carbonized plastic films, or plastic surfaces loaded with highly conductive powdered or solid carbon or graphite.

Battery 28 comprising the next layer may be made up of a group of cells internally connected in series to obtain the desired voltage necessary to obtain the electrophoretic action with the particular polypeptide. Orientation of battery 28 depends on the direction of endosmotic flow which is usually from the anode. With regard to battery 28, it should be noted that any conventional miniaturized battery cells now generally available can be employed, arranged and connected in series to obtain the desired operating voltage. In addition, the technology now exists for batteries made of thin, flexible sheets of an electrically conductive polymer with high surface area relative to its thickness to provide adequate current densities. One such so-called plastic battery is described in "Batteries Today", Autumn 1981, pages 10, 11, and 24. When such a battery is employed, sheets may be layered to place the cells in series, and an effective compromise between number of sheets and surface areas of sheets is achieved by layering them diagonally, as shown somewhat schematically in FIG.

1. Of course, battery selection also depends on such factors as the degree of conformability desired, voltage and current densities required for a specific application, and time of discharge.

In FIG. 1, above battery 28 is electrical contact 32, which preferably is similar in design and material to electrical contact 26 and forms the opposite side of the battery.

Cover 12 encloses all the previously listed layers of drug applicator 10 and is made of flexible, conductive material such as a plastic polymer impregnated with carbon, electrically conductive itself, or metallized on its surface. Insulating material 34 fills the space between the side walls of raised portion 14 and the various aqueous layers containing electrolyte. Suitable insulating materials are polyester, silicones, and any other drug-compatible plastics. Alternatively, a totally insulating cover may envelope all of the working components previously named.

In order for drug applicator 10 to make good contact with and stick to the patient's skin 18 electrically-conductive adhesive 36 is applied under the edge of lip 16. Suitable conducting adhesive materials are those filled with powdered conductors such as carbon or graphite.

It will be seen that the arrangement described above forms a complete electric circuit from one side of battery 28, cover 12, adhesive material 36, skin 18, microporous membrane 22, liquid reservoir 24, and back to battery 28. Also, the reservoir may be divided into separate anode and cathode compartments with an insulator between and the battery in a separate compartment.

The electrical operation of the drug applicator may be carried out in many modes, including that of uniform direct current. The impressed voltage from the power source may be pulsed with a wide variety of pulse width and frequency. A saw-tooth voltage or other types of reversing, sinusoidal, or alternating voltage sources are also within the disclosure of this invention.

The types of batteries and their orientation are disclosed *inter alia* in U.S. Pat. No. 4,557,723 and 4,640,689. The types of circuits which may be employed are also disclosed in various of the above-cited related applications.

Table I shows some of the therapeutic polypeptides with commercial or experimental status. The evolutionary relationships, aminoacid sequences, intra- and inter-relationships, structures, and activity of polypeptide hormones and drugs are generally known and published. The seventh edition of Goodman and Gilman's "The Pharmacological Basis of Therapeutics", Macmillan, New York, 1985 has relevant material. Also see 40th edition of "Physicians' Desk Reference", Medical Economics Co., Oradell, N.J., 1986.

The present invention encompasses the electrolytic transdermal delivery of solutions and suspensions of the numerous polypeptide drugs, hormones, agonists, secretion inhibitors, and regulators of glucose metabolism, water regulation, CNS activity, growth, pain alleviation, blood pressure regulation, cancer alleviation, and other functions for molecules having from about three (cf. thyrotropin releasing hormone) to about 20 (cf. atriopeptin, said to be 21) peptide units. Without being limited by theory, it is believed that the primary mechanism for this electrolytic delivery is by electroosmosis, not ionophoresis, as erroneously subscribed to by the scientific workers in the prior art.

The transdermal patch of the invention may include at least one polypeptide which is LH-RH analog further comprising testosterone. Also, the transdermal patch of the invention may include at least one polypeptide which is LH-RH analog further comprising Flutamide.

In many cases it is preferred to add cosolvent/cosolute molecules with negative Setschenow constants in the polypeptide drugs of the present invention to facilitate their transdermal delivery, as shown in Table II.

Having described the inventive composition of polypeptide, water structure-breaking solute, and aqueous electrolyte and having described the preferred embodiment of the electrolytic drug applicator for transdermal delivery of polypeptides, we now illustrate the invention in the following Examples. These Examples, however, are intended only to illustrate not limit the scope of the instant invention, which may be carried out by other means and still be covered by the teachings of this disclosure.

EXAMPLE 1

This Example illustrates the preparation of small electrolytic transdermal devices with side-by-side reservoirs and electrodes. Another possible design is that of a "matted-photograph" with the drug reservoir anode surrounded by an insulated frame-shaped cathode, as shown in FIG. 1.

The side-by-side reservoirs and electrodes have a rayon gauze next to the skin (Johnson & Johnson Co., New Brunswick, N.J.). Two matted rayon pads 5 cm x 8 cm x 0.5 cm are topped by U-shaped polyester film 0.1 mm thick coated with 0.02 mm layer of conducting graphite paint (Bertek Corp., St. Albans, Vt.) surrounding a central insulator of 0.2 mm Mylar polyester film (duPont Co., Wilmington, Del.). The top surface of the U-shaped graphitized polyester film is connected to a 9 V battery (E1 Power Corp., Santa Anna, Calif.). The periphery of the felted reservoir pads and electrodes plus an insulating band in the gauze base between them is RTV silicone resin (Dow Corning Co., Midland, Mich.). Surrounding the top and side of the device is surgical adhesive tape (Hy-Tape Surgical Hosiery Corp., New York, N.Y.). Each of the reservoirs can hold 6 ml of aqueous fluid.

EXAMPLE 2

This Example illustrates the use of the present invention to deliver therapeutic amounts of an experimental linear polypeptide having about ten peptide units, useful in the treatment of prostate cancer, compound DUA, a variant of leuprolide acetate.

Four male subjects were fitted: two with large drug applicators (9 cm by 13 cm) active area 37 cm² on the chest, subjects A and B, and two with small drug applicators (5 cm by 13 cm) active area 11 cm² on the volar area of the arm, subjects C and D. The large applicators had reservoirs of 0.6 ml; the small 0.15 ml. The solution in the reservoir consisted of 10 percent polypeptide and 1 percent urea.

The battery had a voltage of 9 volts: the current density was 3.5 microamperes/cm² for the preparation time of eight hours. During that time the polypeptide was transdermally transported to the extent of 200 microgr./hr for the large and 60 microgr./hr for the small applicators. Blood analysis shows the amount and activity of the delivered hormone in the hormonal response to the delivery of the drug to the four subjects, as follows:

- A a five-fold increase in 3 hrs.,
- B a four-fold increase in 4 hrs.,
- C a triple response in 5 hrs.,
- D a 12-fold increase within 6 hrs.

This stimulated response of the subjects is typical of a healthy, adult, male receiving therapeutic subcutaneous bolus injection of this hormone.

Model Example 1

This Model Example illustrates the application of the present invention to the delivery of lyppressin (Diapid, Sandoz Co., East Hanover, N.J.) to the bloodstream of the patient.

Eight beagle dogs are employed. All of them are clipped on the back, washed with castille soap, and fitted with the small animal electrolytic patches of Example 1, four of them without batteries.

The drug reservoir of each patch contains 6 ml. Diapid 50 U.S. Patent, 0.185 mg lyppressin/ml. The return reservoir contains 0.9 percent saline. A current regulator is set to deliver 10 $\mu\text{A}/\text{cm}^2$.

Over a period of ten days the total urine output of each dog is measured by standard techniques. It is found that the average total urine volume of the four dogs wearing the powdered device of the present invention is significantly lower than that of the average of the four control dogs.

Model Example 2

This model example illustrates the use of the present invention to delivery vasopressin for the treatment of diabetes insipidus.

Six adult male volunteers each having been diagnosed as diabetes insipidus are tested. Three are fitted with the large-size human patch, as in Example 2, and the three controls are fitted with drug-loaded, identical patches without batteries.

The drug reservoirs of the large electrolyte patches contain 15 ml (300 units) of Pitressin (Parke-aDavis Co., Morris Plains, N.J.). The return reservoir contains 0.9 percent saline buffered to pH 7.2 with ammonium hydrogen phosphate.

Urine samples were taken from each subject three times per day for five days. It is found that the electrical conductivity of the urine of the subjects wearing the powdered Pitressin (vasopressin) electrolyte patches average significantly higher than that of the three control subjects, showing higher electrolyte content.

Model Example 3

This Model Example illustrates the use of the present invention to deliver atrial natriuretic factor, atriopeptin, directly to the bloodstream of patients.

Ten adult female volunteers, diagnosed as having hypertension, are chosen for this experiment. All are fitted with the large-size electrolytic transdermal patch, as in Example 2.

The subjects are fitted with patches of the present invention having a drug reservoir containing 5 ml of a 0.01M solution of atriopeptin in 0.1M sodium perchlorate, a salt with negative Setschenow constants. Five control patches have no batteries. The regulated power source is set to give a current density of about 10 $\mu\text{A}/\text{cm}^2$, delivering about 200 nanograms of drug per hour.

The blood pressure of the ten subjects is measured four times a day. It is found that the average blood

pressure of the five subjects having powdered patches is significantly lower than the average of the five controls.

Although the specification and Examples above describe aqueous media, the present invention is equally applicable to nonaqueous media such as the injectable oils familiar to those skilled in the art, such as glycerine, propylene glycol, benzyl alcohol, and the like.

Many other embodiments of this invention will be apparent to those skilled in the art, but such will be within the scope of Letters Patent based on the following claims.

We claim:

1. A transdermal patch for delivering at least one drug directly to the bloodstream of the patient comprising in combination:

(a) an active ingredient comprising at least one polypeptide having from three to 20 peptide units selected from the group consisting of vasopressin, lyppressin, atriopeptin, oxytocin, desmopressin, a dynorphin, leuprolide, an angiotensin, and mixtures thereof contained in a drug reservoir, and

(b) electrolytic means for transdermal transport of the polypeptide characterized by a current density from about 0.5 microampere/ cm^2 to about 1 milliampere/ cm^2 ,

whereby the skin of the patient is neither irritated nor erythematized.

2. A transdermal patch as in claim 1, wherein the polypeptide comprises vasopressin.

3. A transdermal patch as in claim 1, wherein the polypeptide comprises lyppressin.

4. A transdermal patch as in claim 1, wherein the polypeptide comprises atriopeptin.

5. A transdermal patch as in claim 1, wherein the electrolytic means comprises a battery, an anode, a cathode, said drug reservoir, and barrier means between the electrodes.

6. A transdermal patch as in claim 1, further comprising an aqueous solvent for the polypeptide.

7. A transdermal patch as in claim 6, further comprising a dissociating cosolvent.

8. A transdermal patch as in claim 7, wherein the dissociating cosolvent is selected from the group consisting of urea, alkyl derivatives of urea, guanidine salt, butanol, 2-butanol, water-soluble amides with more than three carbon atoms, sodium and potassium iodide, sodium perchlorate, sodium butyrate, and any other salt with negative Setschenow constants.

9. A transdermal patch as in claim 1, further comprising at least one compound selected from the group consisting of buffering agents, chelating agents, antioxidants, preservatives, and biocides.

10. A transdermal patch as in claim 6, further comprising a semipermeable membrane on the skin side of the drug reservoir.

11. A transdermal patch as in claim 1, wherein at least one polypeptide is LH-RH analog further comprising testosterone.

12. A transdermal patch as in claim 1, wherein at least one polypeptide is LH-RH analog further comprising Flutamide.

13. A transdermal patch as in claim 5, further comprising current controlling means whereby the current density of the patch is from 0.5 microampere/ cm^2 to about 10 microampere/ cm^2 .

14. A transdermal patch for delivering at least one drug directly to the bloodstream of the patient comprising in combination:

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(a) an active ingredient comprising at least one non-ionic polypeptide having from three to 20 peptide units contained in a drug reservoir, and

(b) electrolytic means for transdermal transport of the nonionic polypeptide characterized by a current density from about 0.5 microampere/cm² to about 1 milliampere/cm², whereby the skin of the patient is neither irritated nor erythematized.

15. A transdermal patch as in claim 14, wherein the nonionic polypeptide is selected from the group consisting of vasopressin, lypressin, atriopeptin, oxytocin, desmopressin, leuprolide, a dynorphin, an angiotensin, and mixtures thereof.

16. A transdermal patch as in claim 14, wherein the electrolytic means comprises a battery, an anode, a

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cathode, a drug reservoir, and a barrier means between the electrodes.

17. A transdermal patch as in claim 14, comprising an aqueous solvent for the nonionic polypeptide.

18. A transdermal patch as in claim 14, further comprising at least one compound from the group consisting of buffering agents, chelating agents, antioxidants, preservatives, biocides, and dissociating cosolvents with a negative Setschenow constant.

19. A transdermal patch as in claim 16, further comprising current controlling means whereby the current density of the patch is from about 0.5 microampere/cm² to about 10 microamperes/cm².

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**Absorption and Metabolism of Neurohypophyseal
Hormones, with special reference to
Desmopressin (dDAVP), in Human Tissue and
after various Routes of Administration**

Anne M. Fjellestad-Paulsen



Lund 1996

**From the Department of Clinical Pharmacology
Lund University Hospital
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| Title and subtitle Absorption and metabolism of neurohypophyseal hormones, with special reference to desmopressin (dDAVP), in human tissue and after various routes of administration. | | | |
| Abstract <p>The absorption and metabolism of AVP, oxytocin (OT) and their analogues especially dDAVP, were studied in human tissue, in healthy humans and in children with central diabetes insipidus (DI). When incubated in undiluted intestinal contents AVP was rapidly and completely metabolized whereas an extensive but slower and pH-dependent degradation of dDAVP occurred, especially in the ileum. This degradation was inhibited in a concentration-dependent manner when an enzyme inhibitor was preincubated with intestinal contents. Among the uterotonic peptides C-terminal amides seemed to be more stable than acids. When incubated with gastrointestinal brush border microvilli membranes from the ileum AVP and OT were only metabolized after 180 min and in the presence of reduced glutathione. Synthetic analogues were stable under identical conditions.</p> <p>When AVP, OT and their analogues were incubated for 180 min with human renal microvilli membranes dDAVP was stable while AVP, OT and [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-OT showed different metabolic pathways and they were rapidly metabolized in the presence of glutathione. All peptides except antocin were degraded after 180 min when incubated with crude liver homogenate but not with purified plasma membranes indicating that the proteolytic activity takes place intracellularly.</p> <p>Bioavailability in healthy humans after intranasal (i.n.) and oral administration of dDAVP was found to be 3 % and 0.1 % respectively. The bioavailability of dDAVP after direct application in the stomach, duodenum and the jejunum (0.2 %) was similar to that after swallowing a tablet and was significantly higher than after direct application in the ileum, colon and rectum (0.04 %). No preferential site of absorption was found within the upper segment of the gastrointestinal tract.</p> <p>The bioavailability of dDAVP after direct duodenal application increased 5 times through a simultaneous perfusion of an enzyme inhibitor, demonstrating the effect of proteolytic intestinal enzymes on the absorption of dDAVP. Despite very low bioavailability, oral dDAVP induces a rapid, potent and sustained antidiuresis in DI patients, and is an attractive alternative to the i.n. route of administration. Approximately 20 to 30 times larger doses are needed orally than intranasally, but this does not result in an increase in side effects.</p> | | | |
| Key words absorption, analogues, aprotinin, arginine-vasopressin, bioavailability, desmopressin, diabetes insipidus, gastrointestinal, glutathione, human, intranasal, kidney, liver, metabolism, microvilli brush border membranes, neurohypophysis, oxytocin, pharmacokinetics | | | |
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Signature Anne M. Paulsen

Date April 15, 1996

To Frederik

FLUCTUAT NEC MERGITUR

This thesis is based on studies reported in the following articles, referred to in the text by their Roman numerals:

- I** Metabolism of vasopressin, oxytocin and their analogues in the human gastrointestinal tract. Fjellestad-Paulsen A, Söderberg-Ahlm C, Lundin S. *Peptides* 1995, 16: 1141-1147.
- II** Metabolism of vasopressin, oxytocin and their analogues [Mpa¹, D-Arg⁸]-vasopressin (dDAVP) and [Mpa¹, D-Tyr(Et)², Thr⁴, Orn⁸]-oxytocin (Antocin) by human kidney and liver homogenates. Fjellestad-Paulsen A, Lundin S. *Reg Peptides* 1996. In press.
- III** Pharmacokinetics of l-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers. Fjellestad-Paulsen A, Höglund P, Lundin S, Paulsen O. *Clin Endocrinol* 1993, 38: 177-182.
- IV** Absolute bioavailability of an aqueous solution of l-deamino-8-D-arginine vasopressin from different regions of the gastrointestinal tract in man. d'Agay-Abensour L, Fjellestad-Paulsen A, Höglund P, Ngô Y, Paulsen O, Rambaud J.C. *Eur J Clin Pharmacol* 1993, 44: 473-476.
- V** Bioavailability of l-deamino-8-D-arginine vasopressin with an enzyme inhibitor (aprotinin) from the small intestine in healthy volunteers. Fjellestad-Paulsen A, d'Agay-Abensour L, Höglund P, Rambaud J.C. *Eur J Clin Pharmacol* 1996. In press.
- VI** Central diabetes insipidus in children. Antidiuretic effect and pharmacokinetics of intranasal and peroral l-deamino-8-D-arginine vasopressin. Fjellestad-Paulsen A, Tubiana-Rufi N, Harris A, Czernichow P. *Acta Endocrinol (Copenh.)* 1987, 115: 307-312.

SOME ABBREVIATIONS

| | |
|------------------|--|
| ACTH | adrenocorticotrophic hormone |
| AD | antidiuretic |
| AUC | area under the curve |
| AVP | arginine vasopressin |
| CI | confidence interval |
| Cl | clearance |
| C _{max} | maximum plasma concentration |
| dDAVP | l-deamino-8-D-arginine vasopressin |
| DI | diabetes insipidus |
| EDTA | ethylenediamine tetraacetic acid |
| F | bioavailability |
| GI | gastrointestinal |
| HPLC | high performance liquid chromatography |
| i.n. | intranasal |
| i.v. | intravenous |
| LVP | lysine vasopressin |
| MRT | mean residence time |
| osM | osmolality |
| OT | oxytocin |
| pKa | dissociation constant |
| p.o. | peroral (oral) |
| PEG | polyethylene glycol |
| RIA | radioimmunoassay |
| s.c. | subcutaneous |
| SD | standard deviation |
| SEM | standard error of mean |
| t _{1/2} | half-life of elimination |
| Tc | Technetium |
| T _{max} | time to maximal plasma concentration |
| TRH | thyrotropin-releasing hormone |
| U-osM | urinary osmolality |
| VP | vasopressin |

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INTRODUCTION

Gastrointestinal absorption of intact and biologically active peptides has been extensively studied in the last two decades. Previously it was generally believed that only free amino acids, after complete intestinal intraluminal hydrolysis, could cross the intestinal mucosa and enter the circulation.

However, in 1969 it was first demonstrated in animals (Matthews *et al.*, 1969) and later in man (Adibi and Morse, 1971) that di- and tripeptides could undergo intestinal absorption by active mechanisms and that they were absorbed more rapidly than the free amino acids. These observations have interested scientists working with peptide and protein absorption (Matthews, 1975; Adibi and Kim, 1981; Gardner, 1994), hereditary intestinal deficiencies in amino acid transport (Asatoor *et al.*, 1970; Silk *et al.*, 1975), nutrition (Gardner 1984; Heyman and Desjeux, 1992) and immunology (Baintner 1986; Husby, 1988) and, more recently, peptide and protein drug delivery (Lee *et al.*, 1991).

Due to their poor absorption and susceptibility to enzymatic degradation in the gastrointestinal tract only a few peptides currently employed in therapy are bioavailable after oral absorption such as cyclosporine (Wood *et al.*, 1983; Sawchuck and Awni, 1986), octreotide (Köhler *et al.*, 1987; Fricker and Drewe, 1995), TRH (Yokohama *et al.*, 1984), tetragastrin (Jennewein *et al.*, 1974) and the dipeptide-like angiotensin-converting enzyme inhibitor, moexipril (Grass and Morehead, 1989).

After administration a peptide is required to cross one or more biological barriers between the site of administration and the target receptor. There is sparse information on the gastrointestinal hydrolysis and disposition of such biologically active peptides as the neurohypophyseal hormones and their analogues.

Most of the peptides used in our studies are biologically active and of therapeutic interest. AVP potentiates the responses of ACTH and cortisol to corticotropin-releasing factor (Gillies *et al.*, 1982; Lamberts *et al.*, 1984) and dDAVP is used as long-term substitution therapy in hypothalamic diabetes insipidus and has lately found a number of additional therapeutic applications. Oxytocin is used for the induction of labor and to stimulate lactation. An oxytocin antagonist, antocin (atosiban), has been developed to prevent premature labor (Åkerlund *et al.* 1987; Melin and Trojnar, 1988; Andersen *et al.* 1989; Goodwin *et al.*, 1994) and carbetocin promotes milk ejection in pigs (Barth *et al.*, 1976).

Pharmacology of vasopressin/dDAVP

The principal neurohypophyseal hormones, two cyclic nonapeptides, vasopressin (VP) and oxytocin were identified from extracts of the posterior pituitary gland a century ago (Oliver and Schäfer, 1895; Howell, 1898). In the past three decades, over fifty other peptides have been identified and characterized in the mammalian neurohypophysis. Their concentrations are very low, and their functions almost unknown (O'Donohue and Kiss, 1987).

While no known disease is associated with oxytocin deficiency, central or neurogenic diabetes insipidus (DI), a chronic state of polyuria and polydipsia first described by Frank in 1794 and further characterized by Frank in 1912 (not the same author), is due to absent or impaired production or secretion of vasopressin in the hypothalamic paraventricular and supraoptic nuclei or to blocked transportation to the nerve endings of the posterior pituitary gland (Robinson and Verbalis, 1985).

Vasopressin is synthesized as a peptide pre-prohormone, which is split to produce vasopressin, neurophysin II and glucoprotein (Richter and Schmale, 1985). The gene encoding vasopressin has been isolated and cloned, and is located on chromosome 20, close to the gene encoding oxytocin (Sausville *et al.*, 1985).

Initially, diabetes insipidus patients were treated by substitution therapy with vasopressin given as posterior pituitary extracts administered subcutaneously (Farini, 1913; von den Velden, 1913; Kamm *et al.*, 1928). Subsequently intranasal administration was shown to be effective but limited by simultaneous oxytocic activity in the preparation (Blumgart, 1922). Although synthetic vasopressin is devoid of these effects (Chirman and Kinsell, 1964; Moses, 1964) nevertheless it still has numerous extrarenal actions. These manifest themselves as side effects and, moreover, vasopressin must be given in high doses because of its short plasma half-life.

Apart from its antidiuretic activity (V_2 receptors) vasopressin contracts smooth muscle cells via V_1 receptors in intestinal wall and blood vessels. Moreover, vasopressin exerts a contractile effect on the myometrium and potentiates corticotropin-releasing factor-mediated release of ACTH (Baertschi *et al.*, 1983; Rivier and Vale, 1983). Furthermore, vasopressin increases the release of coagulation factor VIII (Haslam and Rosson, 1972) and tissue plasminogen activity (Manucci *et al.* 1975).

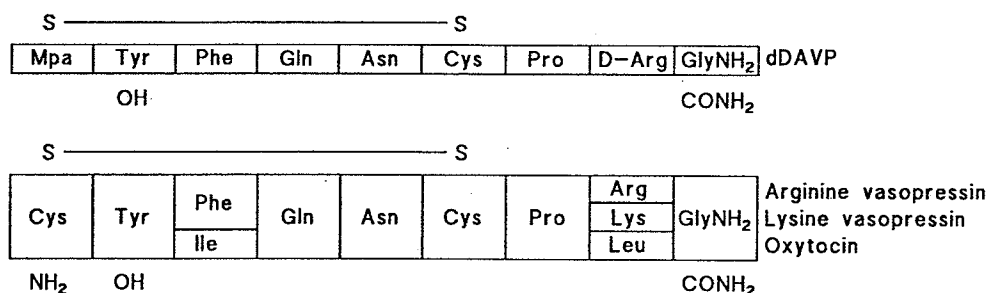


Fig 1. Amino acid sequence of neurohypophyseal hormones (lower panel) and l-deamino-8-D-arginine vasopressin (dDAVP, upper panel). Mpa = β -Mercaptopropionic acid, l-deamino-cystein.

AVP and oxytocin have in common seven of their nine amino acids and a hexapeptide ring closed by a disulphide bond between cysteine residues at position 1 and 6.

When du Vigneaud *et al.* in 1954 discovered the chemical structure of vasopressin (Fig 1), arginine vasopressin (AVP) in humans and most other species and lysine vasopressin (LVP) in the pig, it also became possible to synthesize analogues of the peptide. l-deamino-8-D-arginine vasopressin (dDAVP) was first synthesized by Zaoral *et al.* in 1967 with the aim to increase the ratio between antidiuretic and vasopressor activities by modification of two critical sites of the molecule. dDAVP is distinguished from vasopressin by two structural changes: deamination of the hemicycstine at the N-terminal of the molecule enhances the antidiuretic activity, and substitution of L-Arg at position 8 with the enantiomer D-Arg decreases pressor potency (Fig 1). Additionally, these structural changes have made this V₂ receptor agonist (Jard, 1985) more resistant to enzymatic degradation by amino- and carboxypeptidases and result in prolongation of the antidiuretic effect (Edwards *et al.*, 1973; Seif *et al.*, 1978; Shimizu *et al.*, 1980).

Biological activities and clinical use of dDAVP

Table 1 shows the biological activities of dDAVP in comparison with vasopressin and some analogues. Each molecular modification causes an increase in the ratio between antidiuretic and vasopressor effect. A combination of two modifications results not only in a synergistic increase of the ratio, but also in an important enhancement of the antidiuretic effect of the parent molecule.

Table 1: Biological potencies of vasopressin and related analogues (AD = antidiuretic, P = pressor).

| | Activity | | Ratio |
|-----------------|--------------------|-------------------|-------|
| | AD (IU/ μ mol) | P (IU/ μ mol) | AD/P |
| Vasopressin | 450 | 450 | 1.0 |
| 1-deamino-VP | 1300 | 370 | 3.5 |
| 8-D-arginine VP | 114 | 4 | 28 |
| dDAVP | 1200 | 0.5 | 2400 |

Numerous studies have confirmed the superiority of dDAVP in the treatment of central or neurogenic diabetes insipidus in both adults (Vavra *et al.*, 1968; Andersson and Arner, 1972; Edwards *et al.*, 1973) and children (Aronson *et al.*, 1973; Becker and Foley, 1981) because of its prolonged antidiuretic effect and lack of side effects. The most prevalent route of administration is the intranasal (i.n.). Children and adult patients usually require 5 - 20 μ g dDAVP intranasally once or twice daily (Robinson, 1976) and infants are treated with smaller doses ranging from 1 to 15 μ g once or twice a day (Kauli *et al.*, 1985).

dDAVP is furthermore used diagnostically as a test of renal concentrating function (Aronson and Svenningsen, 1974; Tuvemo, 1978; Curtis and Donovan, 1979). The antidiuretic activity of dDAVP has also resulted in its use in nocturnal enuresis in children (Dimson, 1986; Fjellestad-Paulsen *et al.*, 1987) and in nocturia in adults (Hilton and Stanton, 1982).

Other clinical applications of dDAVP include its use to increase the yield of factor VIII in blood donors (Manucci *et al.*, 1975; Nilsson *et al.*, 1979; Vilhardt and Nilsson, 1981; Mikaelsson *et al.*, 1982), in the treatment of mild hemophilia A and von Willebrand disease (Mannucci *et al.*, 1977; Ruggeri *et al.*, 1982; Lethagen, 1992) and in patients undergoing cardiac surgery to reduce early postoperative bleeding (Salzman *et al.*, 1986; Andersson *et al.* 1990).

In this thesis, the discussion of the pharmacology of dDAVP will be limited to its antidiuretic effect.

Routes of administration of dDAVP

Intravenous and subcutaneous administration

In diabetes insipidus patients dDAVP can be administered as an intravenous (i.v.) bolus dose of 2 μg with optimal antidiuretic effect (Andersson and Arner, 1972; Edwards *et al.*, 1973). Subcutaneous (s.c.) injection of dDAVP may be useful in the treatment of diabetes insipidus patients (Moses *et al.*, 1981) or in the assessment of renal concentration capacity (Tryding *et al.*, 1987). In these studies on the antidiuretic effect, 2 μg given subcutaneously or i.v. were equivalent to 20 μg administered i.n.

Intravenous doses as high as 16 μg dDAVP have been given in one study without causing side effects such as pressor reactions (Andersson *et al.*, 1972) and even with higher doses 0.3 - 0.4 $\mu\text{g}/\text{kg}$ in healthy volunteers or in patients with von Willebrand's disease no or minor adverse reactions (facial flush) have been reported (Lethagen, 1992).

Intranasal administration

The molecular weight of dDAVP (1069) is sufficiently low to permit passage through the nasal mucosa. Therefore, a traditional way of administration of dDAVP is intranasal, either by drops, through a calibrated "rhinyle" catheter or, more recently by a metered-dose nasal spray or a single-dose pipette.

Sublingual administration

An adequate antidiuretic effect of 10 - 12 h with a gelatin-based sublingual lozenge containing 20 μg dDAVP has been reported (Grossman *et al.*, 1980) in diabetes insipidus patients. A similar effect was seen in 18 diabetes insipidus patients with a sublingual tablet containing 30 μg (Laczi *et al.*, 1980).

Oral administration

Vilhardt and Bie showed in 1983, in water loaded conscious dogs, that oral administration of AVP and dDAVP was followed by an antidiuretic response and concomitant increase in urine osmolality. dDAVP was about 10 times as potent as AVP, on an equimolar base.

A dose-dependent antidiuretic effect after oral administration of dDAVP was demonstrated 1984 in hydrated humans (Vilhardt and Bie, 1984) and in 1985 Hammer and Vilhardt showed further the efficacy of oral dDAVP given as tablets in nine adult diabetes insipidus patients.

In an initial dose ranging study we could establish the feasibility and effectiveness of oral treatment in 10 diabetes insipidus children aged 3 to 15.5 years (Fjellestad and Czernichow, 1986).

Other routes of administration

A new method for *transdermal* drug delivery through de-epithelialized skin prepared by suction showed a bioavailability in volunteers corresponding to that after i.v. administration (Svedman *et al.*, 1991; Lundin *et al.*, 1995).

In the lung high amounts of dDAVP were shown to be absorbed in rats and pigs after *aerosol exposure* and *intratracheal* instillation (Folkesson *et al.*, 1990; 1992).

In rats, 25 - 50% of a dDAVP dose that gives an adequate antidiuretic effect after oral administration is effective after *rectal* administration (Saffran *et al.*, 1988). Dogs with diabetes insipidus have been treated with topical *ocular* administration of dDAVP (Schwartz-Porsche, 1980).

Bioavailability of neurohypophyseal hormones and their analogues

Depending on various factors, such as the physiochemical characteristics of the peptide, its molecular weight and the type of delivery system and the galenic formulation, the bioavailability of 10 - 20 amino acid peptides in general, given intranasally, varies between 1 and 12% (Hussain *et al.*, 1985; Sandow and Petri, 1985; Harris, 1986; Su, 1986).

In one of the earliest studies on dDAVP the bioavailability of i.n. dDAVP in DI patients was estimated to be 10 - 20% by comparing the antidiuretic effect after i.n. and i.v. administration (Andersson and Arner, 1972). In a more recent study, using a radioimmunoassay with an antiserum raised specifically against dDAVP, the area under the curve (AUC) of plasma dDAVP after i.n. and oral administration in 6 healthy males was assessed against the plasma clearance after an i.v. dDAVP infusion (Vilhardt and Lundin, 1986a). In this study the bioavailability was 10% after i.n. administration and 1% after oral administration.

Köhler and Harris (1988) compared the i.v., subcutaneous and i.n. administration of dDAVP in man and found a bioavailability as low as 2% after i.n. administration. In this study the i.n. administration was done with a simple dose pipette.

Few studies are available of oxytocin and its analogues. Dawood showed in 1980 increased plasma concentrations of oxytocin in pregnant women and healthy male subjects after oral absorption of oxytocin. Pharmacokinetic properties of antiuterotonic oxytocin analogues have been assessed in rats (Lundin *et al.*, 1993b) and in healthy volunteers (Lundin *et al.*, 1993a) after i.v. administration.

Barriers to absorption

In the following studies, intestinal absorption is defined as the process which describes the uptake into the intestinal mucosa. Bioavailability, on the other hand, is defined here as the fraction of the administered dose to the gut lumen that is absorbed by the intestinal epithelium and reaches the systemic circulation in an intact form.

Numerous factors influence the absorption of a substance, e.g. *physiochemical factors* such as solubility, stability, *pKa* and molecular size and *physiological factors* such as luminal pH, intestinal epithelial permeability and drug metabolism.

Histology

Facing the inside of the small intestine is the *tunica mucosa* with an epithelial cell layer one cell thick, the *lamina propria* and the epithelium of the capillaries.

The luminal surface area is amplified by the *plicae circulares*, large folds of the mucosa with leaf- or finger-shaped villi creating the crypt structure. The area is further magnified by *microvilli* or *brush-border* (Fig 2), increasing the apical surface approximately 14 - 40 fold to a total area of 200 m². The epithelium lining the villi contains different cell types deriving from multipotent stem cells located in the crypts, the absorptive cells or *enterocytes*, mucus-secreting goblet cells, endocrine epithelial cells, and specialized cells such as tuft cells, M-cells and cup cells (Madara and Trier, 1994).

The absorptive cells are highly polarized by a junctional zone that sharply delineates the apical membrane from the basolateral membrane and their general structure resembles other epithelial cells with transport functions, such as colonic or renal tubular cells. The apical plasma membrane has a high protein-to-lipid ratio of 1.7 to 1 (Madara and Trier, 1994). Hydrolytic enzymes and transport proteins essential for degradation and absorption are present in this part of the cell membrane. The epithelial cells are joined at the apical part of the lateral cell membranes through the *epithelial junctions*, *tight junctions* (Fig 2) or "Zonula occludens" (Madara and Trier, 1994).

Proteolytic activity

Orally ingested peptides undergo degradation by pepsin in the stomach (Taylor, 1968) followed by hydrolysis, essentially by the pancreatic proteases in the lumen: trypsin, chymotrypsin and elastase cleave polypeptide substrate at interior sites of the molecule, liberating peptides. These enzymes are called endopeptidases (Keller, 1968).

On the other hand, exopeptidases attack either the carboxyterminal or aminoterminal liberating amino acids (Neurath and Walsh, 1976). Pancreatic juice is rich in carboxypeptidases but contains only a trace of aminopeptidase activity. The hydrolysis of oligopeptidases requires aminopeptidases found in the brush border of the intestinal epithelium (Adibi and Kim, 1981; Kenny and Maroux, 1982).

Tetrapeptides and longer peptides are hydrolyzed by aminopeptidases of the brush border while cytoplasmic enzymes generally hydrolyze di- and tripeptides. Peptidases found in the brush border layer of the intestinal epithelium include aminooligopeptidases, aminopeptidase A and N, angiotensinase, dipeptidase I and III, tripeptidase and carboxypeptidase A (Appel, 1974). Cytoplasmic peptidases are found in the cytosol, such as postproline endopeptidase (Walter and Simmons, 1977), aminotripeptidase, prolidase (Ganapathy *et al.*, 1984) and also

endopeptidase-24.11 (Kerr and Kenny, 1974) and those found in the lysosomes, for example, the cathepsins.

Methods in the study of intestinal transport and metabolism

In vitro systems. Examples are the everted intestinal sac, the Ussing chamber model, isolated membranes of mucosal epithelia and more recently methods for culturing epithelial cell lines on porous filter matrices. The most common cell line consist of human intestinal cells derived from a colon carcinoma, Caco-2 cells. The proteolytic activity in different mucosal tissues can further be assessed by incubating a peptide in mucosal tissue homogenates, isolated microvilli brush border membranes or plasma membranes (Booth and Kenny, 1974) (Study I and II, Fig 3 and Fig 4).

In situ systems. Perfusion disappearance studies can be done in segments or loops of the intestine in animals, the tied loop in anaesthetized animals or the permanently isolated loop in freely moving animals.

In vivo systems. Intestinal mucosal transport can also be investigated *in vivo*. The drug or peptide is administered orally or intragastrically to animals or humans and an indicator of absorption is monitored. Biological activity can be assessed or the rate of absorption and bioavailability can be estimated in blood and/or urine by drug concentration determination and subsequent pharmacokinetic data analysis.

Interpreting the results from these methods is complicated by several factors which influence the absorption from the intestine to the general circulation such as the composition and osmolarity of the luminal contents, the intestinal transit time and renal clearance. Consequently, a combination of techniques may be necessary.

Zimmer *et al.* described in 1981 a method for studies of drug absorption from different regions of the intestinal tract using capsules. This method however necessitates repeated X-ray examinations and there are problems with the loading of the capsule. In two of our studies (IV, V) we have therefore utilized an alternative method, the so called *intestinal perfusion technique* previously utilized by several investigators (Borgström, 1957; Cooper *et al.*, 1966; Miller and Schedl, 1970; Rambaud *et al.*, 1981). A multichannel tube is introduced through the mouth, or, as in our studies, via one nostril and is thereafter positioned at different levels in the gastrointestinal tract. The use of a non-absorbable volume marker makes the technique more quantitative.

Mechanisms for peptide transport across the intestinal epithelium

The concept of peptide uptake after ingestion of dietary proteins or peptides has been reflected on several times during the last 60 - 70 years. For review of the historical aspects of protein and peptide absorption see Matthews 1975, Adibi and Kim 1981 and more recently Matthews 1991.

For many years it was generally believed that only free amino acids could cross the intestinal mucosa before entering the general circulation. In two independent laboratories it was discovered that di- and tri-peptides could undergo transmucosal uptake more rapidly than free amino acids (Craft *et al.*, 1968; Adibi and Phillips, 1968). Subsequently, it was demonstrated that peptides with more than three Gly residues were unsuited for transcellular active transport (Adibi and Morse, 1977). Initially, peptide transport studies were performed with peptides such as Gly-Gly, β -Ala-L-His and Gly-Sar-Sar resistant to intracellular hydrolysis (for review see Matthews, 1975; Matthews, 1991). The transport mechanism was considered to be active when the mucosa - to - serosa flux of these peptides was inhibited by metabolic inhibitors, Na⁺ depletion and hypoxia. The kinetics of these peptides were further shown to be saturable.

Short amino acid chains, up to tetrapeptides, cross the epithelial cell of the brush border membranes mainly by active carrier-mediated transport mechanisms (Fig 2 and Ganapathy *et al.*, 1994). An intestinal peptide transporter has also recently been cloned and was shown to transport peptides not larger than four amino acids (Dantzig *et al.*, 1994; Fei *et al.*, 1994). Larger peptides, on the other hand, are absorbed passively probably via both paracellular and transcellular routes (Gardner, 1994).

An octapeptide composed of D amino acids was found to be absorbed from the intestinal tract in rats drinking high concentrated glucose solutions containing the peptide (Pappenheimer *et al.*, 1994). Other *in vivo* studies have shown similar results suggesting that an active transepithelial flux, of for example, glucose increases the absorption of peptides (Atisook and Madara, 1991; Fricker and Drewe, 1995). This theory of solvent drug-driven solute transport is, however, controversial.

The current view of peptide transport routes and mechanisms across the intestinal epithelium has recently been reviewed by Gardner (1994) and is presented in Fig 2.

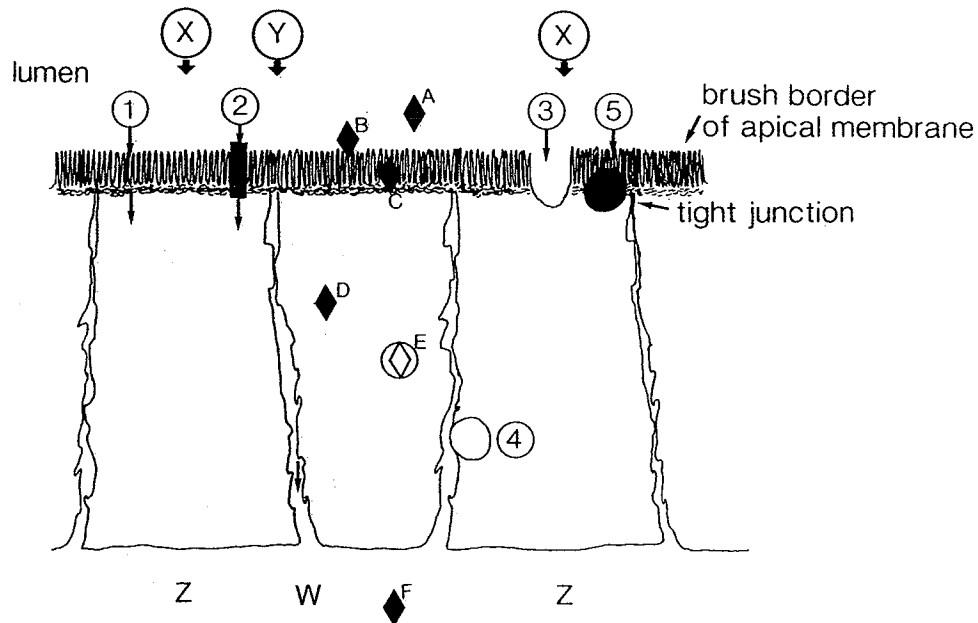


Fig 2. Schematic illustration of a single layer of mucosal epithelial cells acting as a physical barrier between the external environment and the *milieu intérieur*.

Proteolytic activity is indicated by the symbols \blacklozenge : proteolytic enzymes of the pancreas (A: luminal hydrolysis, B: brush border membrane adherent) C, brush border peptidases; D, cytoplasmic peptidases; E, lysosomal proteases; F, post-absorptive peptidase activity.

Amino acid and peptide transport by intracellular route (X) with hydrolysis at sites B and D have been extensively studied and characterized (for review see Ganapathy *et al.*, 1994).

The trans- or intracellular route (X to Z) entails: ① a lipophilic route, often inaccessible to many peptides because of their low lipophilicity, is probably the route for microemulsion absorption enhancers. ② a hydrophilic route through aqueous channels or pores whose existence is still hypothetical.

Routes 1 + 2 are non-saturable, with diffusion-mediated transport. ③ a receptor-mediated endocytotic (RME) route (for large peptides) with formation of phagolysosomes after interaction with lysosomes (E) followed by ④ exocytosis ⑤ a carrier-mediated active and saturable route for small peptides. **The inter- or paracellular route** (Y to W) involves passage through the tight junctions or "Zonula occludens".

Intestinal absorption of neurohypophyseal hormones and their analogues

Oral dDAVP elicited sustained antidiuresis in water-loaded rats (Vavra *et al.*, 1974; Matsui *et al.*, 1985). Oral dDAVP given in drinking water was effective in homozygous Brattleboro rats deficient in AVP, as shown by increase of urinary osmolality (U-osM) and decrease of urinary volume in a dose dependent manner (Kinter and Beeuwkes, 1982). In 1983 Vilhardt and Bie showed (Vilhardt and Bie, 1983) an antidiuretic response in conscious dogs after intragastric administration to AVP and to a greater extent to dDAVP.

Soon thereafter this was confirmed in hydrated humans (Vilhardt and Bie, 1984) and in patients with diabetes insipidus (Hammer and Vilhardt, 1985).

In 1985 Lundin and coworkers described a specific dDAVP radioimmunoassay (RIA), demonstrating the appearance of intact dDAVP in plasma after intragastric administration in rats. Since then, more precise pharmacokinetic studies in animals and in humans on intestinal absorption of dDAVP have been possible.

In an *in vitro* transport study using an everted intestinal sac of the rat, AVP, oxytocin and their analogues were identified by specific RIA methods and by quantitative high performance liquid chromatography (HPLC) (Vilhardt and Lundin, 1986b). The transport rate was highest for dDAVP and it was concluded that the mechanism of transportation was passive diffusion.

Only a limited number of studies describe the oral absorption of oxytocin and its analogues. In 1980 Dawood showed increased plasma concentrations of oxytocin in pregnant women and healthy volunteers after oral administration of oxytocin. In a recent *in vitro* study of oxytocin and analogues in rats, on intestinal transport through the mucosa Lundin *et al.* (1991) showed that these peptides had a higher transport rate across the distal intestinal segment compared with the proximal segment.

This is in accordance with previous *in vivo* studies in rabbits where dDAVP was absorbed to a higher extent from the ileocecal segment of the intestine (Lundin and Vilhardt, 1986). This is also in agreement with two *in vitro/in vivo* studies in the rat (Lundin *et al.*, 1990; Pantzar *et al.*, 1995).

Metabolism and elimination of dDAVP/AVP

Plasma concentrations of dDAVP measured after an i.v. bolus injection in DI patients showed half-lives of elimination ranging between 51 (Edwards *et al.*, 1973) and 158 minutes (Pullan *et al.*, 1978) but these studies were performed with a non specific AVP or LVP antiserum to measure plasma dDAVP. In comparison, half-lives after injection of AVP or LVP were much shorter, 3 to 24 minutes (Edwards *et al.*, 1973; Pullan *et al.*, 1978; Seif *et al.*, 1978; Shimizu *et al.*, 1980). Using a specific RIA with an antiserum against 8-D-arginine vasopressin, Vilhardt *et al.* (1986) found a plasma half-life of 55 min in healthy volunteers after a bolus injection of 5 µg dDAVP.

There is scant information on the metabolic fate of the neurohypophyseal hormones and even less on their synthetic analogues. Most studies have been carried out in experimental animals and practically no data are available in man. The distribution of tritium-labelled AVP (Janaky *et al.*, 1982) and dDAVP (Laszlo *et al.*, 1981) has been investigated in the rat. The radioactivity was found mainly in the small intestine, kidney and liver and, essentially for AVP, also in the adenohypophysis. The main organs for metabolism and elimination for AVP and oxytocin are the liver and the kidney (Walter and Bowman, 1973; Lauson, 1974; Walter and Shlank, 1975). When AVP and OT, labelled with ^{14}C in the glycine residue, were added to isolated perfused rat kidney, the urinary excretion of glycinamide amounted to 60% and 85% of the total radioactivity respectively (Walter and Bowman, 1973).

An *in vitro* inactivation study using rat kidney and liver homogenates showed that 70% of the AVP was inactivated at 30 min, while no more than 20% of the dDAVP was inactivated by the liver homogenate, and very little by the kidney homogenate (Shimizu *et al.*, 1980). Proposed mechanisms of hormone degradation have been forwarded (Pliska and Rudinger, 1976; Walter and Simmons, 1977; Shimizu *et al.*, 1980) and Carone *et al.* showed in 1987 that AVP was metabolized both by isolated brush border membranes and by a cortical lysosomal fraction, which *in vitro* required the presence of reduced glutathione as a cofactor.

AIMS OF THE PRESENT INVESTIGATION

The general aim of this investigation was to study the absorption and metabolism of neurohypophyseal hormones, especially 1-deamino-8-D-arginine vasopressin (dDAVP) in human tissue, in healthy humans and in patients with diabetes insipidus in order to better understand the factors influencing their oral bioavailability.

The specific aims were as follows:

to study the stability of vasopressin, oxytocin and some of their synthetic analogues in human intestinal contents and brush border membranes from the gastrointestinal tract and to evaluate the relative importance of luminal proteolysis.

to investigate the metabolism of vasopressin, oxytocin and some of their analogues by human renal microvilli brush border membranes and human liver membranes.

to compare the pharmacokinetics of dDAVP after different routes of administration in healthy volunteers.

to assess the absolute bioavailability of dDAVP at various sites in the gastrointestinal tract in man and also to identify a possible site of preferential absorption.

to evaluate the proteolytic effect of intestinal enzymes on dDAVP in healthy humans.

to compare the antidiuretic activity after intranasal and oral dDAVP in paediatric central diabetes insipidus patients.

MATERIALS AND METHODS

Subjects

Healthy volunteers

- Study Ia Collection of gastric juice and intestinal contents: 4 healthy male volunteers, 20 - 32 years old.
- Study III Comparison between i.v., subcutaneous, i.n., oral, sublingual and rectal dDAVP: 8 healthy humans (4 females, 4 males), 20 - 57 years old.
- Study IV dDAVP administered to six different regions of the gastrointestinal tract: 6 healthy male volunteers, 24 to 35 years old.
- Study V Absorption of dDAVP from the small intestine with and without an enzyme inhibitor: 6 healthy male humans, 19 to 34 years old.

Diabetes insipidus patients

- Study VI Comparison between intranasal and oral dDAVP: 10 patients (7 boys, 3 girls), 4.5 to 19 years old with central diabetes insipidus of various etiology. Diagnosis of diabetes insipidus was established according to usual criteria (Czernichow *et al.*, 1985): hypotonic polyuria after a 14-h overnight dehydration test and increase of urine osmolality above 800 mosM/kg after an intranasal dDAVP test. The characteristics of the patients are given in Table 2. Six of the patients had previously been treated orally for more than 18 months.

Table 2: Clinical characteristics of 10 diabetes insipidus patients (study VI).

| Initials | Age (years) | Sex | Etiology | Age (years) at onset of symptoms | Polyuria (l/24 hrs) before any treatment | Previous dDAVP dosage (µg) | |
|----------|-------------|-----|---------------------------|----------------------------------|--|----------------------------|-------------|
| | | | | | | intranasal* | oral** |
| FM | 7.6 | M | Craniopharyngioma | 5.5 | NA | 5 + 10 | # |
| AG | 6.5 | M | Histiocytosis | 2.0 | 5.4 | 5 + 10 | 100/200/500 |
| MG | 6.1 | M | Craniopharyngioma | 3.0 | 4.9 | 5 + 5 | 300/ - /500 |
| JP | 18.8 | M | Hypothalamic dysgerminoma | 9.5 | 7.3 | 5 + 5 | 400/ - /200 |
| MS | 4.5 | M | Neonatal idiopathic DI | 0 | 4.9 | 10 + 10 | 200/200/600 |
| CG | 7.3 | M | Histiocytosis | 5.1 | 3.5 | 10 + 10 | # |
| LC | 8.5 | M | Histiocytosis | 6.0 | 2.5 | 5 + 5 | 100/200/500 |
| VDR | 8.0 | F | Idiopathic DI | 2.0 | 8.4 | 10 + 10 | 500/400/900 |
| LM | 7.7 | F | Idiopathic DI | 7.0 | NA | 5 + 10 | # |
| SR | 19.3 | F | Idiopathic DI | 4.0 | NA | 15 + 10 | # |

* twice daily (b.i.d.), ** three times daily (t.i.d.), # not previously treated with oral dDAVP, NA: not available

Peptides and chemicals

The following peptides were used in studies I + II:

Arginine vasopressin (AVP) and analogues, l-deamino-8-D-arginine vasopressin acetate (dDAVP) and desGly-dDAVP. Oxytocin and analogues: [Mpa¹, D-Tyr (Et)², Thr⁴, Orn⁸]-oxytocin (antocin, atosiban), CAP-OH, 8-D-Arg-CAP, desGly-CAP, DesGly-NH₂-CAP, 6-Carba-desGly-CAP and carbetocin (Fig 6). All peptides were synthesized by solid-phase (Ferring Pharmaceuticals) and had a chromatographic purity of > 98%. A wide spectrum proteinase inhibitor, aprotinin, was obtained from Boehringer Mannheim (study I) and from Hoechst laboratories (study V). A reduced form of glutathione was obtained from Sigma and used in study I + II. A specific chymotrypsin inhibitor, chymostatin, was purchased from Sigma (study I).

For s.c. (study III) and i.v. administration (study III, IV, V) the commercially available dDAVP solution containing 4 µg/ml was employed. For intranasal and sublingual administration (study III), a metered nasal spray, 10 µg/dose, was utilized and in study VI the intranasal solution contained 100 µg/ml and was given with a calibrated rhinyle catheter. Oral administration was conducted in study III and VI with tablets containing 200 µg of dDAVP per tablet. For gastrointestinal application lyophilized dDAVP with a chromatographic purity higher than 99% was used (study IV + V). For rectal application (study III) methylcellulose grade 1500 AKLPM was employed as a vehicle.

Laboratory methods and equipment for investigation *in vitro* and *in vivo*

Analytical methods

Plasma-dDAVP was measured after extraction with a method highly specific for dDAVP (Lundin *et al.*, 1985) using an antiserum raised in Dutch rabbits against 8-D-arginine vasopressin. Urinary dDAVP was measured with the same method in study IV (Van de Heyning *et al.*, 1991) and without extraction in study III. Urine and plasma osmolalities were measured using an osmometer (Advanced Osmometer study VI, advanced Microosmometer Mod 3MO study IV, osmometer Svenska labex study III). Peptide degradation products were identified by HPLC employing a Varian apparatus (study I) or a Kontron pump (study I + II) equipped with an autosampler, UV-detector and integrator. Mobile phases were filtered through 0.45 µm filters (Schleicher & Schuell). The bound peptides were eluted isocratically in a system of acetonitrile containing 0.1% trifluoroacetic acid. A flow rate of 1 ml/min was used with UV detection at 220 nm. For amino-acid analysis (study II) fractionated samples from the HPLC of AVP and oxytocin were used without prior reduction or alkylation. Sequence data were obtained on an automated pulsed-liquid sequencer.

Preparation of human brush border and plasma membranes

Collection of human tissue (I, II). Small fractions (10 x 30 mm) of intestinal epithelium were obtained from the stomach, the proximal segment of the jejunum and the distal part of the ileum, from the proximal segment of the colon and from the rectum in patients undergoing surgery for localized tumours.

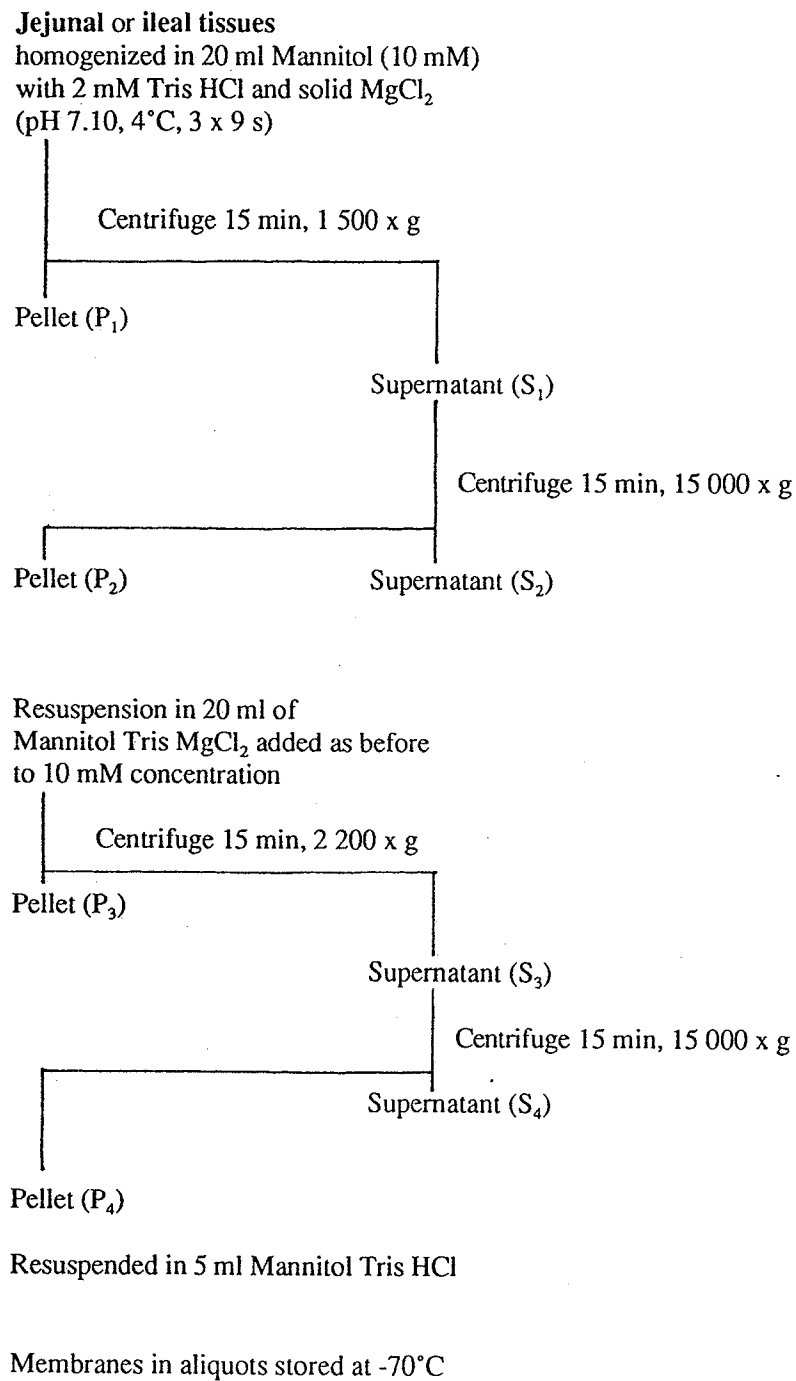
Kidney tissue was obtained from one male patient undergoing nephrectomy because of unilateral adenocarcinoma. The kidney was immediately bisected and chilled on ice after removal. Cortical tissue was dissected free from tumour, medulla, connective tissue and fat. Liver tissue was obtained from a male person who had died in an automobile accident. The tissues were stored frozen at -70°C until required.

Intestinal microvilli brush border membranes (I)

Microvilli brush border membranes from ileum and jejunum were prepared according to a method described by Booth and Kenny (1974) and Fig 3 gives a schematic illustration. Briefly, the frozen intestinal tissue specimens were thawed at room temperature and the mucosal layer was scraped off and immersed in ice-cold isotonic saline.

Solid MgCl_2 was added and the intestinal homogenate was centrifuged at 4°C at $1\,500 \times g$ for 15 minutes and thereafter the supernatant (S_1) was centrifuged $15\,000 \times g$ for 15 min. The microvilli brush border membranes were obtained after centrifuging the $2\,200 \times g$ supernatant (S_3) at $15\,000 \times g$ for 15 minutes. The final pellet (P_4) was suspended in ice-cold Mannitol Tris HCl, homogenized and stored in aliquots at -70°C until used.

Fig 3. Flow-chart for preparation of human intestinal microvilli brush border membranes from jejunum and ileum.



Plasma membranes from stomach and colon were obtained after ultracentrifuging the 2 000 x g supernatant at 100 000 x g twice for 45 minutes.

Plasma membranes from rectum were obtained after centrifugation twice at 48 000 x g for 2 hours.

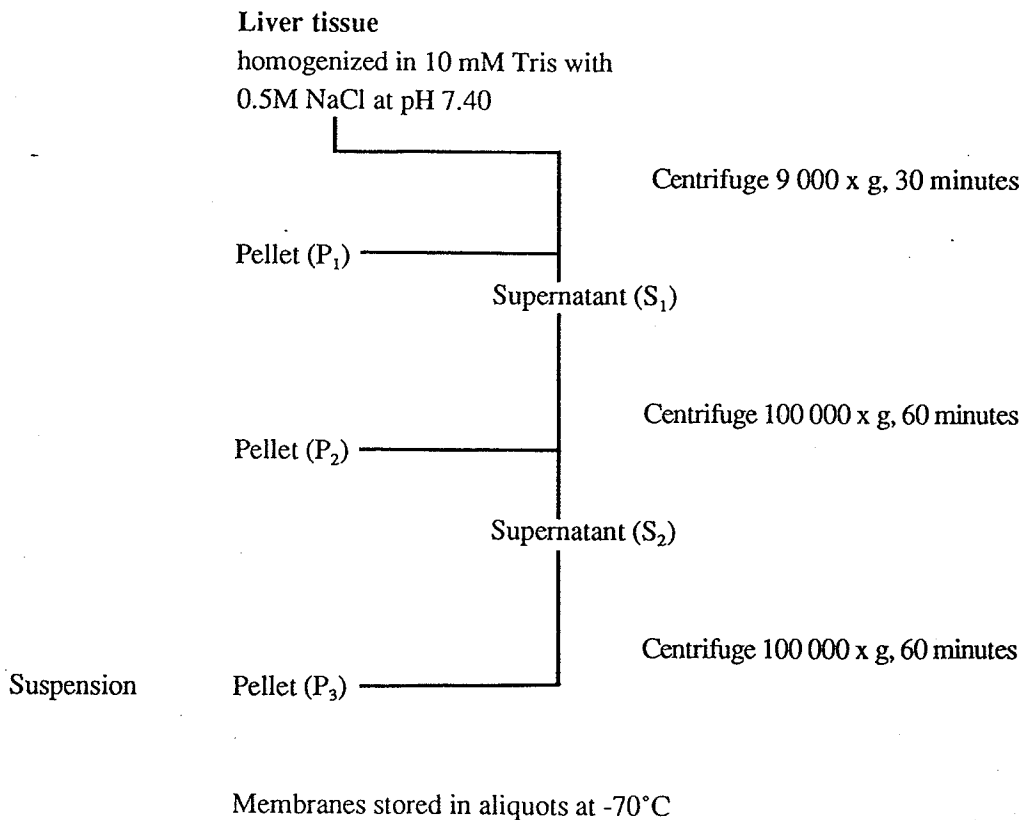
Renal microvilli brush border membranes (II)

Microvilli brush border membranes from human kidney were essentially prepared as described for intestinal brush border membranes (Booth and Kenny, 1974).

Liver preparations (II)

Liver membranes were prepared according to a method described by Postel-Vinay *et al.* (1989). Plasma membranes were obtained after ultracentrifuging the 9 000 x g supernatant at 100 000 x g twice for 60 minutes. A schematic illustration is given in Fig 4. Crude liver homogenate was directly ultracentrifuged twice at 100 000 x g for 60 minutes.

Fig 4. Flow-chart for preparation of human liver plasma-membranes.



Enteral drug application and collection (IV, V)

This technique was a modification of a previously described method (Modigliani *et al.*, 1973). Subjects were intubated via a nostril with a multichannel (study IV) or a triple-lumen channel tube (study V) of polyvinyl chloride (diameter 2 mm).

In study IV dDAVP was directly applied to six distinct sites in the gastrointestinal tract: stomach, duodenum, mid-jejunum, distal ileum, ascending colon and rectum (for the last site a cannula was used). Except for the colon an occluding balloon was placed 2 cm distal to the application point to ascertain the absorption in the chosen intestinal segment.

In study V the tip of the first tube, for the tractor balloon, was positioned after the duodeno-jejunal junction (ligament of Treitz) and that of the second, used for intestinal drug application and perfusion of solutions, was placed in the first part of the duodenum. The tip of the third tube, positioned 30 cm beyond the second, was directed to collect the intestinal juice for measurements of lipase, chymotrypsin and pH. At each session, the positioning of the tube was verified fluoroscopically before drug application (IV + V) and achievement of occlusion and absence of leakage were confirmed by instillation of 1 ml bromosulphthalein as a marker (IV).

Pharmacokinetic calculations

dDAVP kinetics after intravenous infusion were analysed using a two-compartment model in PCNONLIN (Statistical Consultants, Inc. 1986) (version 3.0). In all treatments the area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using the terminal elimination rate.

Clearance, half life of elimination ($t_{1/2}$), absolute and relative bioavailability of dDAVP and mean residence times (MRT) after intravenous and intestinal administrations of dDAVP were calculated using standard formulas (Aronson *et al.*, 1988). The absorption rate (study V) was calculated by a well known method (Loo and Riegelman, 1968). Total clearance after i.v. administration was calculated as dose divided by AUC and renal clearance after i.v. administration as the amount excreted in urine divided by the AUC. The clearances were adjusted using the body weight of each subject.

The maximum concentration (C_{max}) and the time to C_{max} (t_{max}) were the observed values.

Statistical analyses

Statistical evaluation was performed using two-way analysis of variance (ANOVA) study III + IV and with Student's paired t-tests in study V + VI. The level of significance was set at $p < 0.05$.

INVESTIGATIONS AND RESULTS

Metabolism (Study I + II)

Metabolism in human gastric juice and intestinal contents (Ia)

Gastric juice was collected from fasting subjects at 16 h and sampling of duodenal and distal jejunal contents was done 1 h after a standardized meal and sampling of distal ileal contents was done the next morning 2 h after a meal.

Degradation of peptides by gastric juice or intestinal contents was assessed by adding 10 μ l of 10 mM peptide in 0.9% NaCl to 190 μ l of undiluted juice at 37°C. Aliquots were withdrawn at intervals and mixed with acetone and then centrifuged. 10 μ l of the supernatant was analyzed by HPLC.

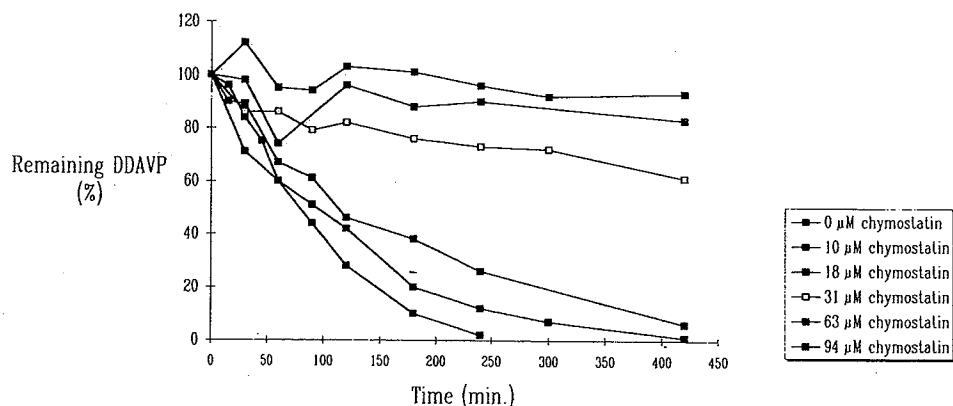


Fig 5. Incubation of dDAVP alone in human intestinal contents from the ileum and with different concentrations of a specific enzyme inhibitor (chymostatin) showing a concentration-dependent inhibition of the degradation of dDAVP.

Results

An important degradation was found to occur in the ileum fraction (pH 6.2) when dissolving dDAVP in the different gastrointestinal juices. Only 50% of the peptide remained intact after approximately 35 - 40 minutes. When contents from the ileum were preincubated with aprotinin a concentration-dependent inhibition of degradation was observed which was also seen with the more specific enzyme inhibitor chymostatin (Fig 5).

Degradation of dDAVP was also found to occur in the duodenal and jejunal fractions after adjustment of the pH to 6.5.

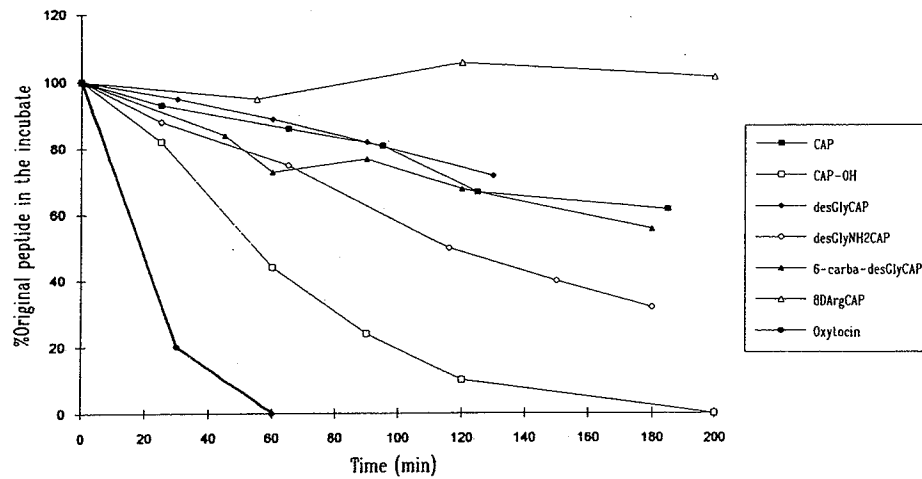


Fig 6. Stability of different uterotonic peptide analogues in human intestinal contents from the ileum indicating that C-terminal amides are more stable than corresponding acids.

AVP was completely degraded within 30 minutes when incubated under the same conditions with intestinal contents from the ileum. Among the uterotonic peptides, oxytocin was degraded almost as rapidly as AVP, and acids were less stable than the corresponding C-terminal amides. The peptide ring, which consists of the first six amino acids with an amide in position six and a sulphur bridge between Mpa¹ and Cys⁶ appeared however to be stable in ileal contents (Fig 6).

Metabolism in brush border membranes from the human intestinal tract (Ib), in human kidney (II) and human liver (II)

Table 3. Degradation of AVP, oxytocin and some analogues in human gastrointestinal microvilli and membrane preparations analyzed by HPLC and expressed as percent of initial value. Time of assessment of degradation is indicated (min). G-, without glutathione; G+, with glutathione; - no breakdown, (+) low breakdown, + breakdown.

| | Stomach | | Jejunum | | Ileum | | Colon | | Rectum | |
|------------|---------|----|---------|--------------------------------|-------|------------------|-------|--------------------------------|--------|------------------|
| | G- | G+ | G- | G+ | G- | G+ | G- | G+ | G- | G+ |
| AVP | - | - | - | + 30% 30' 70% 180' | - | + 85% 180' | (+) | + 40% 30' 50% 180' | (+) | + 30% 180' |
| dDAVP | - | - | (+) | (+) | - | - | - | - | - | + 30% 180' |
| OT | - | - | (+) | - | (+) | + 60% 180' | - | - | - | - |
| Antocin II | - | - | - | (+) | (+) | - | - | - | - | + 25% 180' |
| Carbetocin | - | - | - | - | - | - | - | - | - | - |

Degradation of peptides (AVP, dDAVP, oxytocin, antocin and carbetocin) was assessed by adding 10^{-4} M of peptide to 1 - 10 μ g membrane protein to a final volume of 100 μ l. To half of the incubations, a cofactor (glutathione 10^{-4} M) was added. The incubations, in duplicate, proceeded at 37°C in a rotating water bath and after 0, 30 and 180 min the reaction was stopped by boiling for 5 min followed by centrifugation.

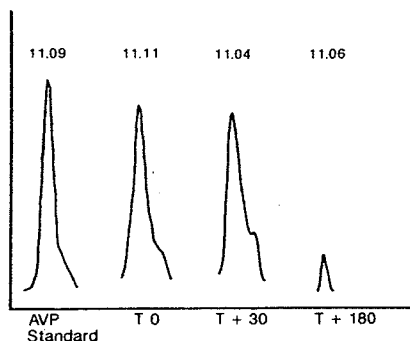


Fig 7. HPLC chromatogram of AVP incubated in human distal ileum mucosa homogenates in the presence of reduced glutathione (10^{-4} M) at 37°C showing a degradation occurring at 180 min. The retention times are indicated on top of each peak [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].

Results

Metabolism in brush border membranes from the human intestinal tract (I)

All membrane preparations showed an increased content of alkaline phosphatase compared to the crude homogenate. Cathepsin B was undetectable, indicating absence of intracellular enzymes.

No degradation of AVP, dDAVP or oxytocin analogues was observed in the plasma membranes from the stomach.

There was a considerable degradation of AVP and oxytocin in the presence of glutathione in the distal part of the ileum (Fig 7) after 180 min. In the presence of glutathione there was a minor degradation of dDAVP and a more extensive degradation of AVP in the homogenized jejunal brush border membrane occurring at 30 min (Table 3).

In the colon only AVP, in the presence of glutathione, was degraded, with just 60% of the initial peptide concentration remaining after 30 min.

AVP, dDAVP and antocin were partially degraded with 70% remaining at 180 min in the rectal mucosa homogenate (Table 3).

Table 4. Degradation of AVP, oxytocin and analogues in kidney microvilli, liver plasma membrane preparations and crude liver homogenate preparations analyzed by HPLC and expressed as percent of initial value. Time of assessment of degradation is indicated (min). G-, without glutathione; G+, with glutathione; - no breakdown, (+) low breakdown, + breakdown.

| | Kidney microvilli | | Liver membrane | | Crude liver homogenate |
|----------|----------------------------|----------------------------|----------------|----|---------------------------|
| | G- | G+ | G- | G+ | |
| AVP | 180' (20%) (+) | 30' (70%) + 180' (100%) | - | - | 30' (15%) + 180' (50%) |
| dDAVP | - | - | - | - | 30' (35%) + 180' (75%) |
| Oxytocin | 30' (80%) + 180' (100%) | 30' (90%) + 180' (100%) | - | - | 30' (5%) + 180' (70%) |
| Antocin | 30' (5%) + 180' (40%) | 30' (70%) + 180' (100%) | - | - | - |

Metabolism in human kidney (II)

All membrane preparations showed high concentrations of alkaline phosphatase compared to the crude homogenate. As summarized in Table 4 the degradation of both AVP and oxytocin was rapid when incubated with renal microvilli membranes in the presence of glutathione (Fig 8).

Due to the rapid metabolism of oxytocin and AVP in the presence of glutathione only two fragments could be sequenced for both peptides (table 5).

Table 5. Amino acid sequences of fragments of oxytocin and AVP obtained after incubation in human kidney microvilli membranes in the presence of 10^{-4} M reduced glutathione. T_r denotes retention time on HPLC.

| | T_r | Peptide sequence |
|----------|-------|--|
| Oxytocin | 4 | Ile-Gln-Asn-Cys |
| | 8 | Cys-Tyr-Ile-Gln-Asn-Cys-Pro [S ————— S] |
| AVP | 6.5 | Cys-Tyr-Phe-Gln |
| | 8.5 | Cys-Tyr-Phe-Gln-Asn-Cys [S ————— S] |

dDAVP remained stable when incubated with renal microvilli membranes. The degradation of antocin was also different with and without reduced glutathione (Table 4). In the absence of glutathione a peak eluted on HPLC which was more lipophilic whereas in the presence of glutathione a more hydrophilic peak was observed. The lipophilic peak was found to coelute with the truncated analogue [Mpa¹, D-Tyr²(Et), Thr⁴, desOrn⁸, desGly⁹]-oxytocin.

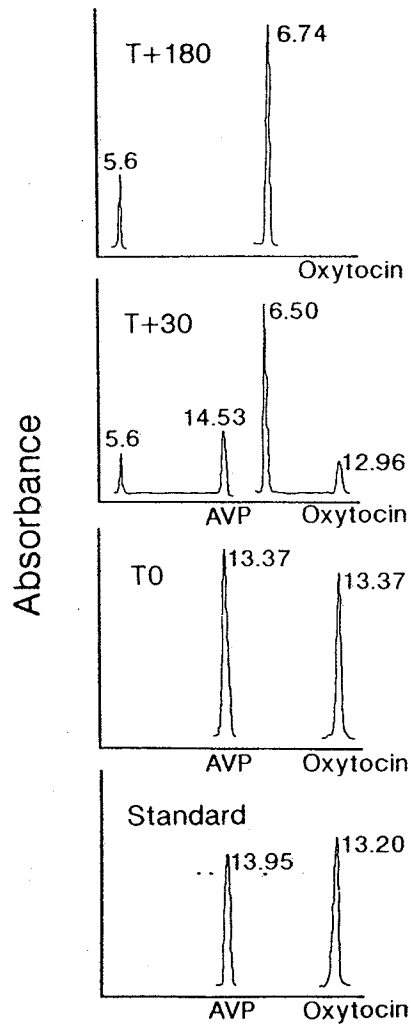


Fig 8. HPLC chromatogram of AVP and oxytocin incubated in human kidney microvilli brush border membranes in the presence of reduced glutathione for 180 min showing a rapid degradation of both peptides and appearance of hydrophilic metabolites.

Metabolism in human liver (II)

There was no degradation of any of the peptides when incubated with liver plasma membranes with or without glutathione. However, when incubating the peptides in crude liver homogenate, degradation occurred with all peptides except antocin (Table 4 and Fig 9).

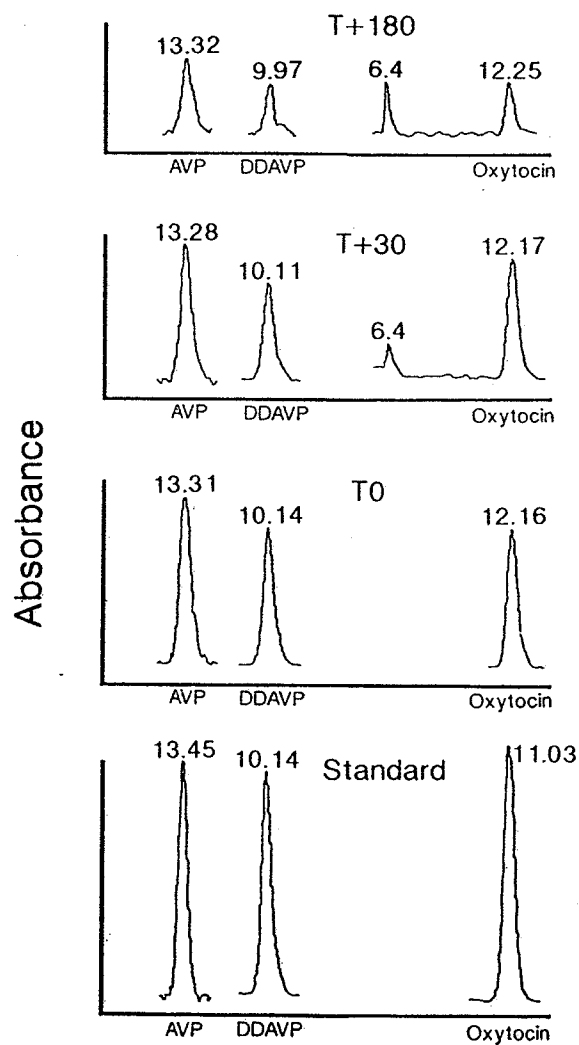


Fig 9. HPLC chromatogram of AVP, dDAVP and oxytocin incubated in human unpurified liver homogenate for 180 min showing a degradation of all three peptides and appearance of a hydrophilic metabolite of oxytocin at 30 and 180 minutes.

Pharmacokinetics (III + IV + V + VI)

Pharmacokinetics in healthy volunteers (III)

dDAVP was administered on six separate occasions, with a washout period of one week, at 8 a.m. to fasting volunteers and a standardized breakfast was served 2 hours later. For s.c. administration 2 μ g of dDAVP was given in the upper arm. For i.v. administration, 2 μ g of dDAVP was diluted with 3.5 ml of NaCl and given over 5 minutes. For i.n. application, 10 μ g was given in each nostril (by spray) with the subjects in an upright position. The total sublingual dose was 20 μ g, with 10 μ g administered at each side of the *lingual frenulum*. 50 μ g dDAVP was administered rectally by syringe. Blood was collected before the administration of dDAVP and at regular intervals during 6 to 8 hours after dose administration.

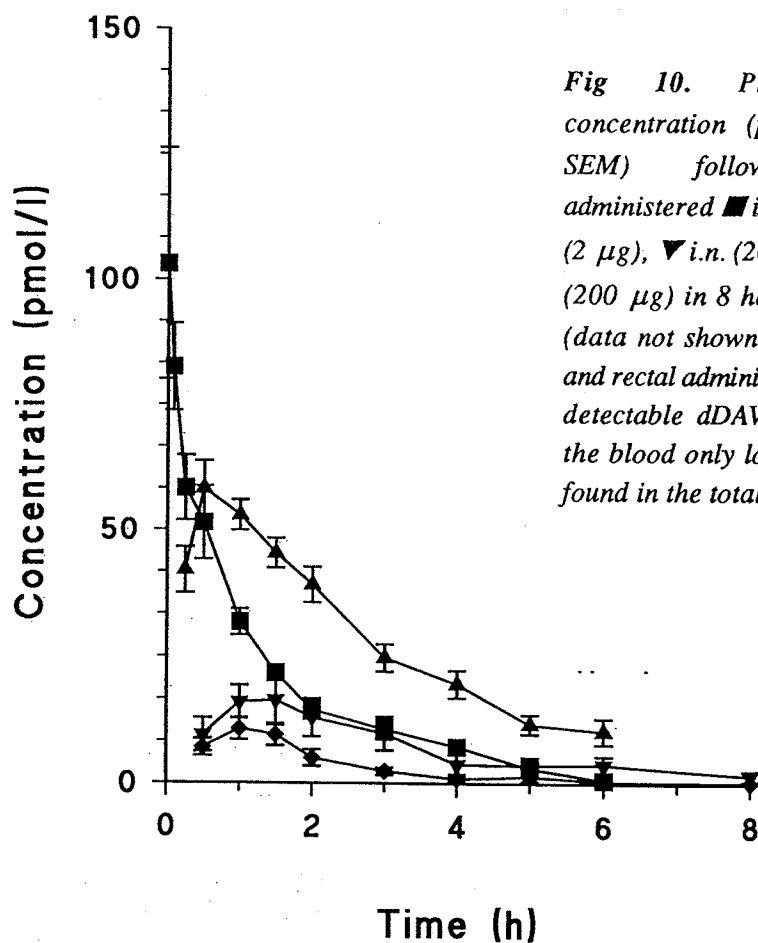


Fig 10. Plasma dDAVP concentration (pmol/l, mean \pm SEM) following dDAVP administered \blacksquare i.v. (2 μ g), \blacktriangle s.c. (2 μ g), \blacktriangledown i.n. (20 μ g) and \blacklozenge p.o. (200 μ g) in 8 healthy volunteers (data not shown from sublingual and rectal administration while no detectable dDAVP was found in the blood only low amounts were found in the total 24-hour urine).

Results

A potent and sustained increase in urine osmolality was observed after all routes of administration, except the sublingual and rectal up to 8 hours after administration.

Accordingly, the 24-hour diuresis in these healthy subjects was somewhat lower (1.0-1.4 l) than normal but no difference was found between the different routes of administration.

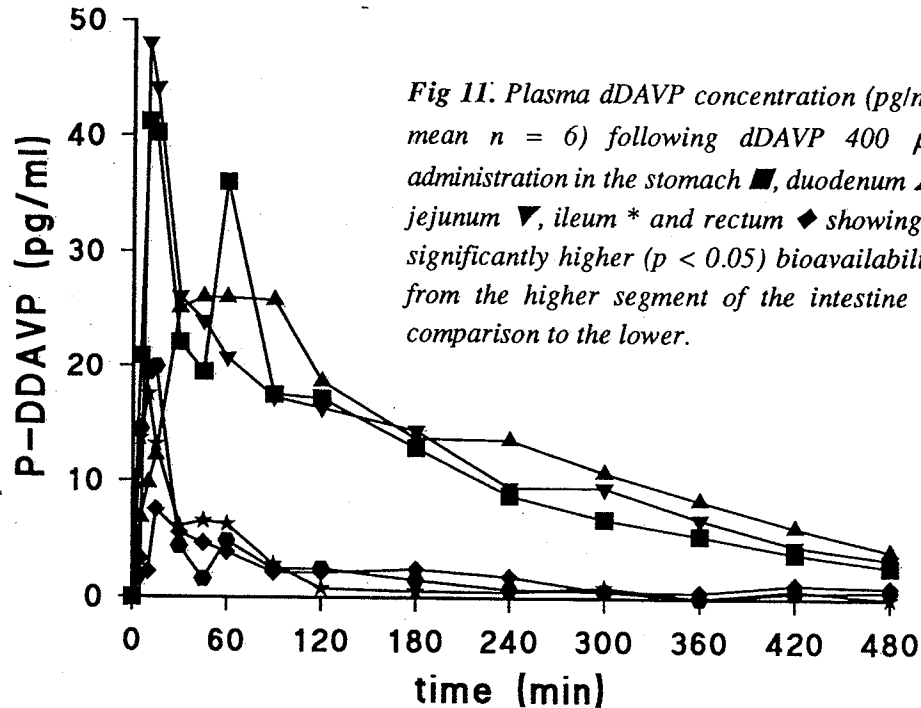
After i.v. administration, the half-life of elimination ($t_{1/2}$) of dDAVP was 78 ± 10 minutes. Using the AUC of dDAVP from the subcutaneous administration as a reference, bioavailability was found to be 3.4% after the i.n. route and 0.1% after the oral route of administration.

After sublingual and intrarectal routes of administration no detectable dDAVP was found in the blood; however, low amounts were found in the total 24-hour urine.

The renal clearance of dDAVP varied from 1.19 to 3.04 ml/min/kg body weight with different routes.

Absolute bioavailability after oral absorption (IV)

Each volunteer participated in seven study sessions, separated by at least 48 h. For calculation of the AUC after i.v. administration, a bolus injection of 4 μ g dDAVP was given at 09.00 h after an overnight fast. In all other sessions 400 μ g dDAVP was dissolved in 2 ml NaCl 0.9% and was given by the appropriate route at 9 a.m. followed by rinsing the tube with 2 ml NaCl. The occlusive balloon was deflated 1.5 h after drug application and a standardized meal was given 1.5 h later.



Results

U-osM remained elevated for at least 8 - 12 h following dDAVP administration in these normally hydrated volunteers irrespective of the site of application, although normal water intake was allowed 3 h after drug administration. Concomitantly, diuresis remained decreased for 12 h. The 24 h diuresis was lower (0.696 - 1.28 l) than normal in healthy volunteers (Carver and Paska, 1961). After i.v. administration, the half-life of elimination ($t_{1/2}$) of dDAVP was 60.0 ± 14.7 min and plasma clearance was 1.7 ± 0.7 ml \times min⁻¹ \times kg⁻¹. Renal clearance was 53.6 ± 19.9 ml \times min⁻¹ or 0.8 ± 0.3 ml \times min⁻¹ \times kg⁻¹.

Individual AUCs and bioavailabilities of dDAVP after application at various sites in the gastrointestinal tract are shown in Table 6 and the mean plasma dDAVP concentrations are shown in Fig 11. The bioavailability was significantly higher from the three upper than from the three lower regions ($P < 0.05$) of the gastrointestinal tract.

| Route of delivery (dose dDAVP)/subject | 1 | 2 | 3 | 4 | 5 | 6 | Mean | SD |
|---|--------|--------|--------|--------|--------|--------|--------|--------|
| AUC (pg \cdot min/ml) | | | | | | | | |
| Intravenous (4 μ g) | 36.700 | 66.800 | 28.000 | 64.700 | 25.400 | 26.050 | 41.300 | 19.400 |
| Stomach (400 μ g) | 13.000 | 1.240 | 7.190 | 3.250 | 3.000 | 8.640 | 6.060 | 4.380 |
| Duodenum (400 μ g) | 5.540 | 4.450 | 6.048 | 2.710 | 8.770 | 16.300 | 7.300 | 4.830 |
| Jejunum (400 μ g) | 3.420 | 27 | 6.450 | 10.400 | 6.370 | 10.600 | 6.210 | 3.720 |
| Ileum (400 μ g) | 1.400 | 1.390 | 840 | 190 | 535 | 2.130 | 1.080 | 701 |
| Colon (400 μ g) | 1.620 | 334 | 640 | 83 | 3.100 | 546 | 1.050 | 1.130 |
| Rectum (400 μ g) | 1.760 | 325 | 607 | 189 | 1.660 | 2.490 | 1.170 | 931 |
| f (%) | | | | | | | | |
| Stomach | | | | | | | | |
| Duodenum | 0.35 | 0.02 | 0.26 | 0.05 | 0.12 | 0.33 | 0.19 | 0.14 |
| Jejunum | 0.15 | 0.07 | 0.22 | 0.04 | 0.35 | 0.62 | 0.24 | 0.22 |
| Ileum | 0.09 | < 0.01 | 0.23 | 0.16 | 0.25 | 0.41 | 0.19 | 0.14 |
| Colon | 0.04 | 0.02 | 0.03 | < 0.01 | 0.02 | 0.08 | 0.03 | 0.03 |
| Rectum | 0.04 | 0.01 | 0.02 | < 0.01 | 0.12 | 0.02 | 0.04 | 0.04 |
| | 0.05 | 0.01 | 0.02 | < 0.01 | 0.07 | 0.10 | 0.04 | 0.04 |

Table 6. AUC and bioavailability (f) of dDAVP directly applied to various sites in the gastrointestinal tract in 6 healthy volunteers.

Bioavailability with an enzyme inhibitor (V)

Each volunteer participated in three study sessions separated by at least 48 hours. For calculations of AUC after i.v. administration a bolus injection of 4 μ g of dDAVP was given at 9 a.m. after an overnight fast. In the two other sessions 500 μ g of dDAVP were administered as in study IV. A standardized breakfast was given 5 - 10 min after drug application and a standardized meal 4 hours later. In one session, a solution of PEG, a non absorbable marker (pH = 6.5, osmolality = 305 m osM/kg) was given as a continuous enteral administration by a motor driven syringe starting 30 min before the dDAVP application and during a further 5.5 h at a constant rate of 5 ml/min. In the other session, a solution of PEG and aprotinin was administered enterally at a constant rate of 5 ml/min and during a further 5 hours.

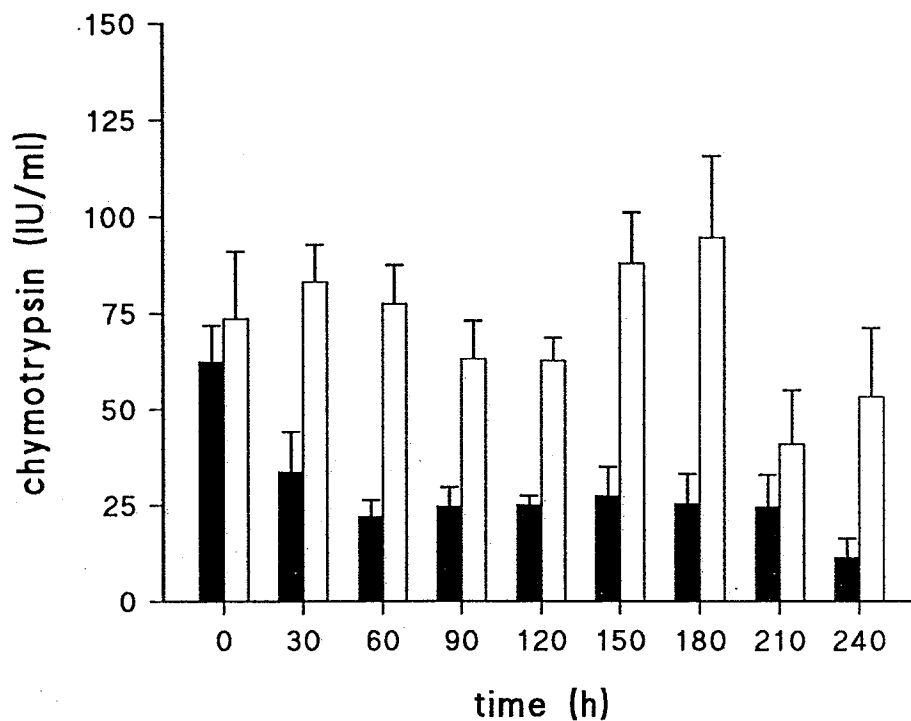


Fig 12. Mean intraluminal activity of chymotrypsin during a 4 hour duodenal perfusion with (■) or without (□) aprotinin after direct application of dDAVP in the duodenum in 6 healthy volunteers ($p < 0.001$).

Results

The intestinal lipase activity after 4 hours was not modified by aprotinin. In contrast, the chymotrypsin activity was significantly decreased after infusion of aprotinin (Fig 12). The median hydrogen ion concentration of the intestinal juice at the jejunal aspiration site was not influenced by aprotinin (pH 5.73 without and 5.75 with aprotinin). After i.v. administration the $t_{1/2}$ of dDAVP was $1.56 \text{ hrs} \pm 0.2$ and plasma clearance $1.24 \pm 0.16 \text{ ml/min} \times \text{kg}$. Bioavailability (F) after duodenal administration of dDAVP + aprotinin was 0.46% (range 0.28 - 0.84) in comparison with 0.09% (range 0.05 - 0.14) after duodenal administration of dDAVP alone ($P < 0.0148$). AUC and F were statistically higher after duodenal administration of dDAVP with aprotinin in comparison with dDAVP alone (Fig 13 and Table 6). However, the absorption rate constant for dDAVP did not differ irrespective of administration with or without aprotinin.

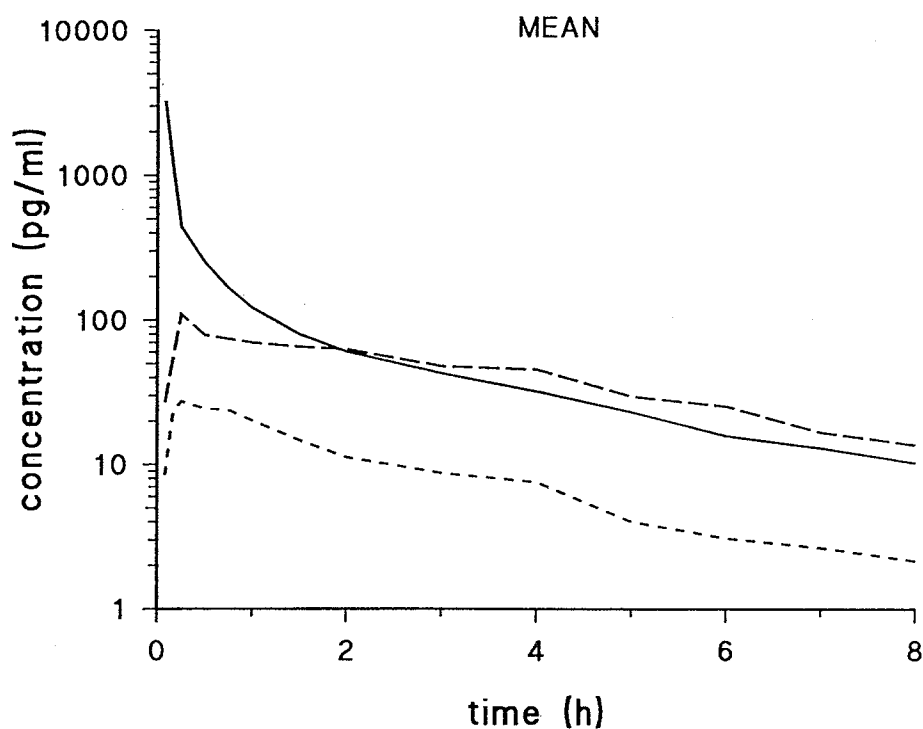


Fig 13. Mean plasma concentrations (pg/ml) of dDAVP after an i.v. bolus injection of 4 µg dDAVP (—), 500 µg dDAVP with aprotinin (---) and 500 µg dDAVP alone (-·-) given in the duodenum in healthy volunteers (n = 6).

Pharmacokinetics in diabetes insipidus patients (VI)

Prior to the investigation, treatment with i.n. or oral dDAVP was discontinued for 36 h. The patients received the different doses in the same order, day 1 and 2 they were given 10 and 20 µg of intranasal dDAVP and day 4 and 5 they received 200 and 400 µg tablets, respectively. The children did not receive any evening dose and were without any treatment on day 3. All had normal breakfast and fluid intake *ad libitum* was permitted throughout the study.

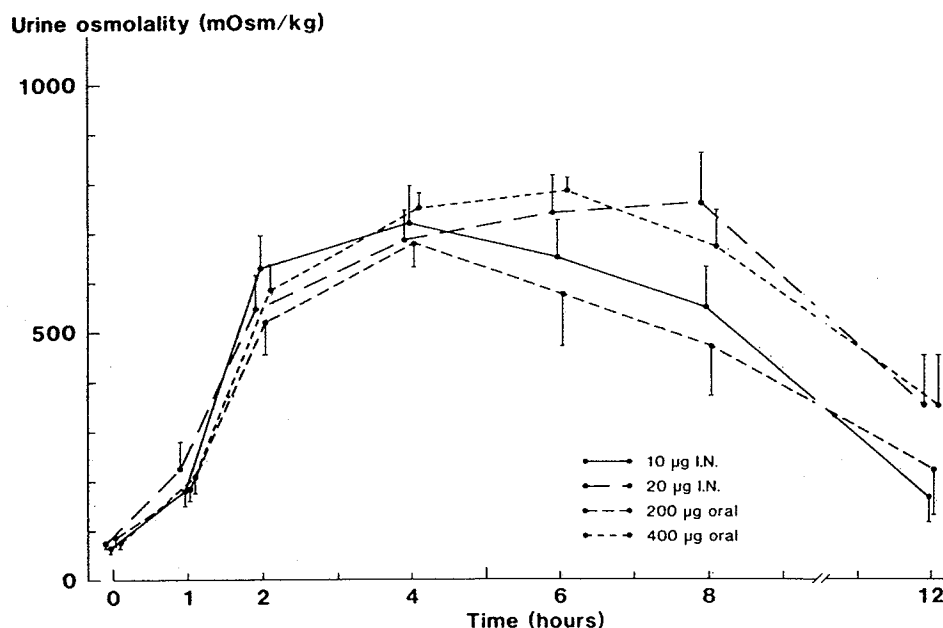


Fig 14. Urine osmolality (mosM/kg, mean \pm SEM) in ten diabetes insipidus patients aged 4.5 to 19 years following intranasal dDAVP (10 μ g \bullet — \bullet ; 20 μ g \bullet — \bullet) and oral dDAVP (200 μ g \bullet --- \bullet ; 400 μ g \bullet --- \bullet).

Results

The U osM increased rapidly in all cases during the second hour after administration, and in a similar manner after i.n. and oral administration, up to 8 h after administration (Fig 14). At 8 h, the difference in U osM between 10 and 20 μ g of i.n. dDAVP was significant ($P < 0.01$). The maximum U osM was higher after the largest doses tested (20 μ g i.n. and 400 μ g p.o.), but the difference was not significant. A difference was however observed in the duration of effect. After 12 h the mean U osM was still above 350 mosMol/kg with 20 μ g of dDAVP i.n. and 400 μ g p.o., whereas with 10 μ g i.n. and 200 μ g p.o. the U osM was 167 and 221 mosmol/kg, respectively (Fig 14). Free water clearance (FWC) showed no difference between doses or routes of administration of dDAVP and remained negative for 7 to 8 h. The mean (\pm SD) duration of U osM remaining above 400 mosMol/kg was 7.4 h \pm 3.0 and 9.0 h \pm 3.2 after 10 and 20 μ g of i.n. dDAVP and 7.2 h \pm 3.3 and 8.8 h \pm 2.3 after 200 and 400 μ g p.o., respectively. A statistical difference was found between 10 and 20 μ g of i.n. dDAVP ($P < 0.05$).

Maximum plasma concentration of dDAVP was obtained for the different doses after 39 to 50 min and thereafter the concentration decreased following first order kinetics for both the i.n. and oral routes of administration.

The calculated plasma β -half-life of dDAVP ranged between 3.3 and 4.0 h and was not different after oral or i.n. dosing.

With regard to the AUC the difference between the i.n. and the oral routes was significant in the 2 groups 10 μ g i.n./200 μ g p.o. - 20 μ g i.n./400 μ g p.o. ($P < 0.05$).

DISCUSSION

Intestinal absorption of neurohypophyseal hormones and their analogues (I)

Investigations I and II were undertaken in order to further study whether the neurohypophyseal hormones and some of their synthetic analogues are hydrolysed by luminal proteolysis, by brush border membrane-bound peptidases in the intestine and in the kidney and by liver plasma membranes in man.

Four possible cleavage sites of the neurohypophyseal hormones have been identified (Walter and Simmons, 1977): 1) Cleavage of the Cys¹-Tyr² peptide bond by a cystine aminopeptidase (vasopressinase, oxytocinase) found in the plasma during pregnancy, 2) reduction of the disulphide bond by thiol: protein disulphide oxidoreductase, 3) cleavage between Pro⁷-Leu⁸ (oxytocin) and Pro⁷-Arg⁸ (AVP) by a postproline endopeptidase and 4) release of the terminal glycnamide residue by trypsin (AVP) or chymotrypsin (oxytocin).

As indicated by the antidiuretic effect in hydrated rats, the activity of AVP was completely eliminated when preincubated with trypsin or α -chymotrypsin while dDAVP's activity remained intact when incubated with trypsin probably because trypsin cleaves carboxyl groups of basic natural amino acids L-arginine and L-lysine bonds of peptide chains (Matsui *et al.*, 1985) and dDAVP has a D-Arg in position 8. When preincubated with α -chymotrypsin, dDAVP lost 50% of its activity (Matsui *et al.*, 1985). Already in the 1950's pressor hormones were found to be susceptible to trypsin cleavage while oxytocin was found to be resistant (Tuppy, 1968). HPLC measurements after incubation in diluted 1:100 rabbit ileum juice showed metabolism of AVP whereas dDAVP was stable (Matuszewska *et al.*, 1988). In enterokinase-activated pig pancreatic juice Lundin *et al.* 1989 found that AVP was rapidly degraded within 5 minutes while dDAVP was stable for 60 min.

In our study of luminal proteolysis in man (Ia), while AVP was completely degraded within 30 min, a slower, pH-dependent degradation of dDAVP was seen in undiluted intestinal contents from different parts of the small intestine especially in the distal part of the ileum. When dDAVP was preincubated with the enzyme inhibitor aprotinin a concentration-dependent inhibition was seen. A similar inhibition was obtained with a more specific enzyme inhibitor (chymostatin) (Fig 5, p 31).

Among the uterotonic peptides tested C-terminal amides seemed to be more stable than acids. In one analogue, substitution of sulphur by a carba group at position six of the ring structure did not increase stability against gastrointestinal enzymes. Ornithine at position 8 increased the resistance to tryptic cleavage while a D-Arg residue at this position completely abolished the degradation. Finally, deamination at position 1 and substitution of L-Tyr with

D-Tyr (Et) at position 2 in the ring structure of the oxytocin molecule increased resistance to chymotrypsin (Fig 6, p 32).

A recent study showed an extensive degradation in concentrated intestinal contents in the rat of several vasopressin analogues including dDAVP except for [Mpa¹, D-Tyr(Et)², Ile³, Val⁴, D-Arg⁸] vasopressin (Lundin *et al.*, 1994). On the other hand, this analogue has an antidiuretic effect which is considerably reduced compared with dDAVP.

Bioavailability of three oxytocin analogues increased after diversion of pancreatic juice in pigs (Lundin *et al.*, 1995) but as the peptides hardly were susceptible to enzymatic degradation other explanations were forwarded for the increased absorption such as pancreatic juice acting directly or indirectly on the gastrointestinal epithelium. Conflicting results from some studies may be explained by the fact that degradation more easily occurs in undiluted, or more highly concentrated, intestinal contents.

Earlier studies have shown that AVP is degraded while dDAVP remains intact after incubation with ileal mucosa (Matuszewska *et al.*, 1988; Lundin *et al.*, 1989). Recently, however, dDAVP was found to be slowly metabolized in rat intestinal mucosa homogenates (Ungell *et al.*, 1992).

In study I b the neurohypophyseal hormones were slowly degraded by intestinal microvilli membranes and colonic and rectal plasma membranes when reduced glutathione 10⁻⁴M was added to the incubations (Table 3, page 32). The synthetic analogues were stable.

The major enzymatic barriers to intestinal absorption of AVP, oxytocin and their analogues are therefore present in the intestinal contents and not in the mucosa, which however constitutes a major physical barrier to peptide transport.

Numerous studies have tried to better characterize the permeability of the small intestine and transport mechanisms to AVP, oxytocin and many of their analogues by *in vitro* (Vilhardt and Lundin, 1986b; Lundin & Artursson, 1990; Lundin *et al.*, 1991; Pantzar *et al.*, 1993) and *in vivo* (Lundin *et al.*, 1990; 1994; 1995; Pantzar *et al.*, 1995) transport systems. No transport maximum and no competitive inhibition could be observed, the highest uptake was in the distal part of the ileum and no correlation was found between bioavailability, hydrophobicity and absorption rate although dDAVP had the highest absorption rate in comparison to other analogues. These studies clearly favor the existence of a passive paracellular absorption for dDAVP. dDAVP probably does not affect its own transport rate and the existence of an active, receptor-mediated endocytosis mechanism is unlikely (Pantzar *et al.*, 1995).

Although no specific receptors for either AVP or oxytocin have been found on the apical side of the enterocyte, cellular uptake by endocytosis with subsequent hydrolysis in lysosomes may however occur for these natural peptides (Carone *et al.*, 1987; Folkesson *et al.*, 1988; Lutz *et al.*, 1991).

Metabolism of neurohypophyseal hormones and their analogues in the kidney (II)

In study II, when vasopressin, oxytocin and their analogues were incubated for 180 min in human renal microvilli brush border membranes, dDAVP was stable whereas AVP, oxytocin and [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-oxytocin (antocin) showed different metabolic pathways according to the presence or the absence of reduced glutathione (Fig 8 and Table 4).

Glutathione is removed in the process of membrane purification and is required as a cofactor in *in vitro* experiments (Carone *et al.*, 1987). *In vivo* glutathione may play a role for the initial splitting of disulphide bonds by reduction (Rabenstein and Yeo, 1994). When incubated with pig kidney microvilli-membranes without glutathione, AVP was resistant to hydrolysis while oxytocin was slowly metabolized by cleavage of the Pro⁷-Leu⁸ bond (Stephenson and Kenny, 1987).

In our study, AVP was almost stable when incubated without reduced glutathione while oxytocin was metabolized. This could be the result of the action of the membrane-bound enzyme endopeptidase 24.11 (Stephenson and Kenny, 1987) which cleaves hydrophobic peptide bonds such as Pro⁷-Leu⁸ (Turner, 1987) and not due to the post-proline enzyme whose location is cytoplasmic (Walter and Simmons, 1977).

Even antocin, which is structurally more similar to AVP at the C-terminus, was found to be cleaved at this site. The peptide sequencing (Table 5) identified fragments from the peptide ring structure suggesting that several enzymes must have been involved. Their action was facilitated by initial splitting of the Cys¹-Cys⁶ disulphide bond.

dDAVP was not metabolized by renal microvilli-membranes neither with nor without reduced glutathione. The presence of D-Arg in position 8 gives the molecule an overall greater stability in addition to deamination in position 1. In healthy volunteers plasma clearance of dDAVP is 3 - 4 times lower (Vilhardt *et al.*, 1986, studies IV, V) than that of the more modified analogue antocin (Lundin *et al.*, 1993).

The differences in plasma clearance may result from factors other than enzymatic stability such as binding to blood constituents and to the rate of excretion in the kidney and in the liver. In fact Lundin *et al.* (1993) found in healthy volunteers that 28.7% of an i.v. dose of antocin and 65.4% of dDAVP were excreted in urine which is in accordance with data from study III. In a similar way, a rather low 24-h urinary excretion was also found for antocin in a pharmacokinetic study in rats (Lundin *et al.*, 1993b).

Degradation of AVP, oxytocin and antocin was rapid when incubated with renal microvilli membranes together with reduced glutathione while dDAVP was stable.

Metabolism of neurohypophyseal hormones and their analogues in the liver (II)

Whereas dDAVP was stable after incubation with renal microvilli membranes, 75% of the peptide was inactivated by human liver crude homogenate at 180 min (Study II) which is more extensive than that observed in rat liver homogenates (Shimizu *et al.*, 1980). 50% of AVP was inactivated under the same conditions and 70% of oxytocin. This latter peptide showed a hydrophilic metabolite at 30 min. All peptides except antocin were degraded in crude liver homogenate but not in purified plasma membranes indicating that the proteolytic activity takes place intracellularly rather than in the membranes (Fig 9, p 36).

Metabolism by intracellular enzymes has been observed for several peptides such as TRH (Scharfmann *et al.*, 1989), cholecystokinin (Gores *et al.*, 1986) and GRF (1-29)NH₂ (Boulanger *et al.*, 1992) and an aminopeptidase like enzyme metabolizing oxytocin has been isolated and partially characterized in primate liver microsomes and lysosomes (Sri Krishna and Kanagasabapathy, 1989).

It is possible that neurohypophyseal hormones are degraded in the hepatocytes after receptor-mediated endocytosis and transport to lysosomes for metabolism (Lutz *et al.*, 1991). Liver perfusion experiments in the rat (Rabkin *et al.*, 1980) and in the pig (Lundin *et al.*, 1991) demonstrating an hepatic clearance of AVP and dDAVP support these findings. Degradation of dDAVP in the liver likely takes place at its C-terminal as the dDAVP antiserum is specific for this region, and since no immunoreactive fragments were observed (Lundin *et al.*, 1991). In cirrhotic patients with poor liver function, the metabolic clearance rate of AVP was found to be reduced and its half-life prolonged under steady-state conditions (Solis-Herruzo *et al.*, 1992).

Oxytocin, AVP and dDAVP were metabolized when incubated in crude liver homogenates indicating that the proteolytic activity takes place intracellularly rather than in the plasma membrane.

Pharmacokinetics after intravenous, intranasal and oral delivery (III)

The intranasal route is a well characterized alternative to injection as a route for administration of peptides. Hepatic first-pass metabolism is by-passed, absorption into the systemic circulation can occur rapidly through the highly vascularized surface of the nasal mucosa and the proteolytic activity is lower in the nasal mucosa than in the gastrointestinal tract (Parr, 1983; Zhou and Li Wan Po, 1990).

The intranasal permeability to peptides may be significantly increased by different types of absorption enhancers (Zhou and Li Wan Po, 1991). Nevertheless, this route is less reliable,

since swollen or inflamed nasal mucosa is unsuitable for local administration, particularly for chronic administration in substitution therapy.

The oral route offers a more convenient and compliant method of drug administration. l-deamino-8-D-arginine vasopressin or dDAVP is one of the very few peptides of therapeutic interest that can cross the gastrointestinal mucosa and enter the general circulation.

In one of the first studies on dDAVP, comparing the antidiuretic effect after i.n. and i.v. administration of dDAVP, the biological activity of dDAVP given i.n. to DI patients was estimated to be 10 - 20% of the i.v. route (Andersson and Arner, 1972). Vilhardt and Lundin (1986a) found a bioavailability of 11.3% after 20 µg given intranasally and 0.7% after a 200 µg tablet (Table 7). However in this study the AUC of plasma dDAVP after i.n. and oral administration was assessed against the plasma clearance after an i.v. dDAVP infusion in another study. They also found that oral administration of tablets of 100 and 200 µg of dDAVP was followed by a dose dependent and sustained antidiuretic effect in 6 hydrated volunteers. This has been confirmed by several authors (Table 8) in healthy volunteers and adult and pediatric DI patients (Cunnah *et al.*, 1986; Fjellestad and Czernichow, 1986; Westgren *et al.*, 1986; Williams *et al.*, 1986).

In our pharmacokinetic study (III) in 8 healthy volunteers the bioavailability of dDAVP was 3.4% after 2 x 10 µg given intranasally and 0.1% after 200 µg given orally. The bioavailability after oral administration seems lower than earlier reported (Table 7). In a pharmacokinetic study (Köhler and Harris, 1988) where the haematological effects of dDAVP were evaluated the bioavailability of 300 µg given i.n. was however 2%. This could partially be explained by the fact that the intranasal administration in our study (III) was made with a metered-dose nose spray and in the above mentioned study with a single-dose pipette.

A nasal spray pump device gives a higher absorption rate than nasal drops (Harris *et al.*, 1986). Various methods of i.n. delivery were compared in healthy volunteers by measuring the deposition and clearance of dDAVP in the nasal cavity using gammascintigraphy. dDAVP solutions containing ^{99m}Tc-labelled human serum albumin were administered i.n. as a spray, using metered-dose pumps, or as drops, using a rhinyle catheter or a single-dose pipette. The sprays were deposited mostly anteriorly, small amounts were cleared slowly into the nasal pharynx. Conversely dDAVP administered as drops was deposited mainly posteriorly and cleared very rapidly. A considerable part was swallowed immediately. Assessment of plasma levels showed that absorption of dDAVP was 2 to 3-times higher with the spray than with the drops. Furthermore it has been shown that by optimizing concentration, volume and technique of administration by the nasal route a significant increase of the bioavailability can be reached (Harris *et al.*, 1988).

| Ref | Number and Subjects | Route of administration | Dose dDAVP | Cl in ml per min per kg | AUC pg x h/ml | F % | t _{1/2} min | C _{max} pg/ml | t _{max} min | Renal clearance ml/min/kg |
|--|-----------------------|---|---|--------------------------|---|--------------------|---|------------------------|-------------------------------------|--|
| Vilhardt et al. 1986 | 8 Volunteers | IV bolus followed by infusion (3.5 hrs) | 5 µg 1538 pg/min/kg | 2.6 | | | 55 | | | |
| Aunsholt et al. 1986 | 8 Uraemic patients | Infusion (0.5 hr) | 0.3 µg/kg + 50 ml NaCl | 1.4 (range 0.5 - 1.7) | | | 200 (99 - 661) | | | |
| Vilhardt/ Lundin 1986a | 6 Hydrated volunteers | IN (rhinyle) PO (tablet) PO (tablet) | 20 µg 100 µg 200 µg | | | 11.3 1.0 0.7 | | | 60 - 120 | 0.514 |
| Fjellestad-Paulsen et al. 1987 (Study VI) | 10 Children with DI | IN (rhinyle) IN (rhinyle) PO (tablet) PO (tablet) | 10 µg 20 µg 200 µg 400 µg | | 157 ± 109 240 ± 202 122 ± 67 260 ± 380 | | 196 ± 30 228 ± 43 238 ± 109 158 ± 64 | 41 76 33 104 | | |
| Fjellestad-Paulsen et al. 1993 (Study III) | 8 Volunteers | IV (5 min) SC IN (spray) Sublingual Oral (tablet) Rectal (syringe) | 2 µg + 3.5 ml NaCl 2 µg 20 µg 20 µg 200 µg 50 µg | | 126 ± 11 208 ± 18 65 ± 16 - 26 ± 5 - | | 78 ± 10 | | - 41.4 60.0 - 71.4 - | 1.71 1.30 3.84 - 1.19 - |

| Ref | Number and Subjects | Route of administration | Dose dDAVP | Cl in ml per min per kg | AUC pg x h/ml | F % | t _{1/2} min | C _{max} pg/ml | t _{max} min | Renal clearance ml/min/kg |
|--|---------------------|--|--|-------------------------|---------------|-------------|----------------------|------------------------|----------------------|---------------------------|
| d'Agay-Abensour et al. 1993 (Study IV) | 6 Volunteers | IV bolus | 4 µg | 1.7 ± 0.7 | 688 ± 323 | | 60 ± 15 | | | 0.8 ± 0.3 |
| | | In situ: Stomach | 400 µg | | 101 ± 73 | 0.19 ± 0.14 | | | | |
| | | Duodenum | 400 µg | | 122 ± 81 | 0.24 ± 0.22 | | | | |
| | | Jejunum | 400 µg | | 104 ± 62 | 0.19 ± 0.14 | | | | |
| | | Ileum | 400 µg | | 18 ± 12 | 0.03 ± 0.03 | | | | |
| | | Colon | 400 µg | | 18 ± 119 | 0.04 ± 0.04 | | | | |
| | | Rectum | 400 µg | | 20 ± 16 | 0.04 ± 0.04 | | | | |
| Fjellestad-Paulsen et al. 1996 (Study V) | 6 Volunteers | IV bolus | 4 µg | 1.24 ± 0.2 | 76 ± 33 | 0.09 ± 0.04 | 93 ± 12 | | | |
| | | Duodenum (In situ) Duodenum (In situ) | 500 µg 500 µg + Aprotinin Perfusion | | 371 ± 147 | 0.46 ± 0.21 | | | | |

Table 7. Comparison of pharmacokinetic characteristics in healthy volunteers and in patients with diabetes insipidus (DI) after administration of dDAVP by various routes of administration (Cl = clearance, F = bioavailability).

We showed further (III) that absorption after oral doses was approximately 30 times lower than intranasal. This is in accordance with our pharmacokinetic study in pediatric DI patients (Study VI) where a ratio of 1:20 was found between the intranasal and oral route. This ratio is confirmed by clinical experience when DI patients change from intranasal to oral administration.

In the different pharmacokinetic studies (III, IV, V) half-lives of elimination of dDAVP after i.v. injection were 78, 60 and 90 minutes respectively which is in accordance with other studies where also a specific antiserum against dDAVP was used (Vilhardt *et al.*, 1986) (Table 7). When high doses of dDAVP have been administered intravenously or intranasally in the hematological indications, higher half-lives have been reported (Lethagen *et al.*, 1987; Köhler and Harris, 1988; Harris *et al.*, 1988).

The urinary excretion of dDAVP after the various routes (III) is more extensive than previously reported (Vilhardt and Lundin, 1986a) when expressed as a percentage of the amount absorbed (65 vs 16.4% after oral administration).

Site of absorption (IV)

Different sites of absorption in the human gastrointestinal tract were investigated in order to identify a possible site of preferential absorption as previously described in animals. In rabbits, a preferential area of absorption of dDAVP seems to be located in the ileocaecal region (Lundin and Vilhardt, 1986) where the absorption was 10 times higher in comparison to the stomach, the mid-part of the ileum or the colon and twice as high as in the duodenum.

In an *in vitro* study in rats, where the absorption was studied in everted small intestinal sacs, the transport of dDAVP was found to be most extensive in the distal (ileal) part of the small intestine (Lundin *et al.*, 1990). Recently three other *in vitro* studies in rats have confirmed an increased distal intestinal permeability to oxytocin and vasopressin analogues (Lundin *et al.*, 1991; Pantzar *et al.*, 1993; 1995). In this latter study one experiment was also made *in situ* in closed small intestinal loops where a 2-fold higher absorption was observed. Explanations for the differences in peptide transport rates observed in animals between proximal and distal intestinal segments have been forwarded (Vilhardt and Lundin, 1986b; Lundin *et al.*, 1990; 1991; Pantzar *et al.*, 1993; 1995). Higher distal paracellular permeability due to more frequent pores or increased paracellular water flux is possibly the main reason, and, in addition, a decreased regional proteolytic capacity may influence the degree of absorption.

There may be species differences between human and animals since in our study in healthy volunteers (IV) no preferential site of absorption was found in the upper segment of the gastrointestinal tract (Study IV, Fig 11, Table 6). All the animal studies concerning the absorption in the distal part of the ileum concern only rodents: rats and rabbits.

Bioavailability after direct application of dDAVP in the stomach, in the duodenum and in the mid-jejunum (0.2%) was comparable to bioavailability after swallowing a tablet (Study III). Surprisingly, the bioavailability after application in the distal part of the ileum was much lower than expected (0.03%).

The reasons why we did not find increased absorption in the distal part of the small intestine in healthy subjects are obscure. The regional proteolytic activity could be slightly higher as shown by the degradation of dDAVP when incubated in intestinal contents from the ileum (Study Ia). Although human studies are more delicate to perform than animal studies, in our study (IV) the distal part of the ileum (and other sites) was studied separately as intended, and peak plasma concentrations (C_{max}) of dDAVP occurred before the balloon was deflated. Several other factors may explain these differences. The intestinal absorptive surface area decreases in the distal direction (Fisher and Parsons, 1950) and the intestinal permeability to non peptides generally has been shown to decrease from the duodenum to the terminal ileum (Loehry *et al.*, 1973).

For other orally bioavailable peptides of therapeutic interest the preferred absorption site is the upper gastrointestinal tract (Jennewein *et al.*, 1974; Sawchuck and Awni, 1986; Kohler *et al.*, 1987; Grass and Morehead, 1989).

The intragastric absorption was unexpectedly high (0.2%) and higher than observed in the rabbit (Lundin and Vilhardt, 1986). AVP and also dDAVP have shown to be resistant to pepsin degradation (Thorn, 1959; Matsui *et al.*, 1985) but the lack of villi and microvilli in the stomach with subsequent reduced absorption area would on the contrary have resulted in low absorption.

Sublingual administration (III)

No antidiuretic effect or detectable plasma-dDAVP was found in our pharmacokinetic study in healthy volunteers (III) after $2 \times 10 \mu\text{g}$ sublingual administration with a metered spray although two previous studies had described this route of administration. A gelatin-based sublingual lozenge containing $2 \times 10 \mu\text{g}$ of dDAVP effected an adequate antidiuretic effect in 6 patients with cranial diabetes insipidus (Grossman *et al.*, 1980). A sublingual tablet containing $30 \mu\text{g}$ gave a 12 h antidiuresis in 18 DI patients (Laczi *et al.*, 1980). However, in these studies plasma dDAVP was not measured. In our study the doses may have been too low, the mode of administration inappropriate, and some amount could have been swallowed.

In an additional experiment, two subjects were given $60 \mu\text{g}$ sublingually with a syringe. Even so, we did not find any detectable dDAVP in blood although the urinary osmolality was slightly increased compared to the previous experiment and detectable amounts of dDAVP were found in 24-hour urine. A 3-month-old child with DI and abnormal nasopharyngeal

anatomy was equally well controlled with the same dose of dDAVP intranasally and sublingually (2 µg/24 h) (Kappy and Sonderer, 1987).

The bioavailability after buccal administration is generally poor: both large peptide drugs such as insulin (Nagai, 1985), as well as smaller peptides such as protirelin (TRH analogue) and LHRH analogue (Merkle *et al.*, 1985) show disappointing results.

Colonic absorption (I, IV)

There is scant information on the permeability of the colonic mucosa to peptide and protein drugs, especially in humans. The proteolytic activity is much less in the colon than in the small intestine but on the other hand there is a smaller surface available for absorption and the colonic epithelium is considered to be "tight" in comparison to the ileal epithelium which has a "tight" (villus) and a "leaky" (crypt) epithelium arranged in parallel (Madara and Trier, 1994). Colonic transit and pH can be variable and the higher numbers of microorganisms especially anaerobics may potentially lead to a more rapid drug degradation. Development of delivery systems that target peptide release specifically in the colon have been described (Kompella and Lee, 1992).

In 1986 Saffran *et al.* reported a study in rats where polymer encapsulated insulin was targeted to be delivered in the large intestine and where enteric bacteria cleaved the protective polymer allowing absorption of insulin. This elicited a hypoglycemic response, however with large variations in time. Nanocapsules have been used to prolong the absorption of lipophilic drugs through the intestinal mucosa. Intragastric administration of polyalkyl cyano acrylate nanocapsules containing insulin was followed by hypoglycaemia by the second day after administration lasting 6 to 20 days depending on the dose given (Demge *et al.*, 1988).

In study IV, after direct application of an aqueous solution of 400 µg dDAVP in the ascending colon in humans the bioavailability was low (0.04%) but similar to that observed in the distal ileum and in the rectum (Table 6, Fig 11). *In vitro*, AVP was found to be slowly degraded by purified colonic plasma membranes in the presence of reduced glutathione (Table 3) whereas the analogues dDAVP and antocin were stable (I).

Rectal administration (I, III, IV)

Rectal administration is of interest mainly because first-pass metabolism is reduced, the systemic absorption is rapid and contact with digestive fluids is avoided. The rate of absorption is not influenced by ingestion of food.

Although low proteolytic activity and neutral pH favor breakdown of polypeptides in the lower segment of the intestinal tract, the uptake of macromolecules is low (Zhou and Li Wan Po, 1991). Saffran *et al.* showed in 1988 that natural vasopressin peptides (AVP and LVP) are more effective after rectal application than after intragastric delivery in rats but the doses

are still 20 times higher than after s.c. administration. Furthermore, in the same study, 25 - 50% of an orally active dDAVP dose gave an adequate antidiuretic effect when given by rectal route. In addition, the dose could be reduced 20 times when given together with an enhancer, 5-methoxysalicylate. To our surprise no dDAVP was measurable after 50 µg given intrarectally together with a vehicle (methylcellulose) by syringe (III). Therefore in an additional experiment we gave 100 µg intrarectally directly without additive. No detectable dDAVP in blood was found but urinary osmolality was slightly increased and low amounts of dDAVP were found in 24-hour urine, in contrast to the previous experiment.

In a second study (IV) a measurable absorption was found after rectal administration. Bioavailability of 400 µg dDAVP given intrarectally with a cannula (0.04%) was much lower than that in the upper segment of the gastrointestinal tract (0.2%) in healthy subjects (Table 6, Fig 11) but similar to that in the distal part of the ileum.

In addition, in an *in vitro* study (I), there was a slight degradation of AVP and dDAVP in the presence of reduced glutathione in purified plasma membrane preparation from human rectum (Table 3).

Influence of an enzyme inhibitor on gastrointestinal absorption (I, V)

The simultaneous s.c. administration of the protease inhibitor aprotinin together with insulin increased the plasma levels of insulin in volunteers (Owens *et al.*, 1988). In contrast, co-administration of aprotinin did not significantly alter the absorption of insulin in nasal absorption studies in rats and rabbits (Deurloo *et al.*, 1989; Morimoto *et al.*, 1991) possibly because of lower proteolytic activity in nasal mucosa.

When AVP and dDAVP were each co-administered intranasally with aprotinin to rats no increase of the antidiuretic effect was found (Morimoto *et al.*, 1991). Aprotinin has been administered in the duodenum without causing any side effects (Dlugosz *et al.*, 1983), and aprotinin is not destroyed by intestinal proteolysis (Frey *et al.*, 1967) or absorbed by the intestinal mucosa (Markwardt, 1978). Saffran *et al.* (1979, 1988), reported enhanced activity of AVP, LVP and dDAVP after simultaneous oral administration of aprotinin in rats. The effect of aprotinin on the oral activity of dDAVP was however inconsistent.

In our first study (I) we were able to show a pH- and concentration-dependent inhibition of the proteolysis of dDAVP when aprotinin was preincubated with contents from the ileum. As the bioavailability of orally ingested dDAVP is very low we wanted to evaluate the proteolytic effect of intestinal enzymes on the absorption of dDAVP in healthy volunteers (V).

Bioavailability of dDAVP with a simultaneous perfusion of aprotinin in the duodenum increased five times in this study (Fig 13). The possible effects on digestion of dietary proteins by the co-administration with a protease inhibitor are however unknown especially

if one considers a long-term substitution therapy with dDAVP and aprotinin. One consequence of this finding is however that the dDAVP tablets in patients should perhaps be taken fasting and not during meals. Higher plasma concentrations of dDAVP were observed when dDAVP tablets were given to fasting hydrated normal volunteers but no difference in biological effect was seen when the tablets were taken with or without food (Rittig *et al.*, 1995).

In a recent study in pigs Lundin *et al.* (1995) show enhanced intestinal absorption of three oxytocin analogues after direct application in the duodenum. The bioavailability ranged between 0.38 et 0.67% before and increased by a factor of 5 to 10 after diversion of pancreatic juice.

In study V the bioavailability of dDAVP after direct duodenal application increased five times through a simultaneous intestinal perfusion of an enzyme inhibitor demonstrating the importance of the proteolytic effect of intestinal enzymes on the intestinal absorption of dDAVP.

Pharmacokinetics in diabetes insipidus patients (VI)

In 1922 Blumgart demonstrated that intranasal administration of pituitary extracts was as effective as the subcutaneous route and since then the i.n. route has been the most common in diabetes insipidus patients. Intranasal dDAVP has been the treatment of choice in hypothalamic DI patients for almost thirty years. The onset of action occurs within one hour and the duration of effect is between 6 and 24 hours. Thus, dDAVP is given once or twice daily (Robinson, 1976). Difficulties may arise during upper respiratory infections and, especially in small children and in patients with poor vision, for technical reasons.

Motzfeldt showed already in 1918 that polyuria in a DI patient was controlled by ingestion in the evening of 2 - 7 fresh bovine pituitary glands. This was confirmed by Rees and Olmsted (1922) who administered desiccated posterior pituitary lobe in coated oral capsules. It was not until 1985 however, that oral dDAVP given as tablets was shown to be efficient in adult DI patients (Hammer and Vilhardt, 1985). The oral doses were approximately ten times higher than the previous intranasal dDAVP doses, but the patients had a slightly increased diuresis of 2 - 3 l per 24 hours (Table 8).

In a dose ranging study the effectiveness of oral dDAVP was thereafter shown in ten pediatric DI patients (Fjellestad and Czernichow, 1986). In an initial study which lasted for six days in the hospital the children received increasing doses of oral dDAVP, from 12.5 to 600 µg. Doses as small as 12.5 µg had effects on renal concentrating capacity and a 200 µg dDAVP tablet produced antidiuresis with a duration of 8 to 12.5 h in different patients (Table 8). The interindividual variability was considerable and two patients needed higher doses.

| Ref | Subjects | No | Mean age (yrs) (range) | Treatment period | Mean total 24 h dose of dDAVP | Dose regimen | Diuresis/24 h during treatment |
|---------------------------------|---------------------------|----|---------------------------|---------------------|--|--|-----------------------------------|
| Hammer & Vilhardt 1985 | DI patients (adults) | 9 | 30.5 (21 - 53) | 6 days | 277 µg | 100/100/100 in most cases; twice daily in 2 patients | 2 - 3 |
| Fjellestad & Czernichow 1986 | DI patients (children) | 10 | 8.8 (3 - 17.5) | 6 days | 525 µg | 150 - 500 µg twice daily | 1.26 |
| | | | | 6 months | 850 µg | in two patients = 100 - 200 b.i.d. in all others t.i.d. morning and midday 100 - 300 µg evening 400 - 800 µg | 1.07 |
| Cunnah et al. 1986 | DI patients (adults) | 9 | 33.8 (5 - 67) | 3 - 6 months | 294 µg | t.i.d. | 1.38 |
| Westgren et al. 1986 | DI patients (children) | 7 | 12.6 (4 - 18) | 6 months | 550 µg | from 100 µg x 2/24 h to 400 µg x 3/24 h | |
| Fjellestad-Paulsen et al. 1993a | DI patients (children) | 7 | 12.7 (7 - 21) | 3.5 years | 906 µg | | 1.7 |

Table 8. Comparison of oral dDAVP doses given to adult or pediatric diabetes insipidus patients during different time periods (b.i.d. = twice daily, t.i.d. = three times daily).

No significant increase in biological effect was seen with doses exceeding 200 µg (up to 600 µg). Diuresis started to decrease 15 - 30 min after intake of a 200 µg tablet, reaching a nadir after one to two hours. In parallel, urinary osmolality increased rapidly to reach the maximum above 900 UosM/kg in 2 - 3 hours.

During the dose ranging study a logarithmic relationship between dose and duration of antidiuresis was found ($P < 0.001$). The patients were discharged with doses ranging between 150 to 500 µg twice daily (525 µg/24 h).

Following the controlled conditions in the hospital, the mean daily dose for most children had to be increased during the first weeks at home. Thereafter, the tablets were given three times a day in order to decrease the total amount of dDAVP. After 6 months the patients were treated with a mean daily dose of 850 µg (Table 8) divided into 3 doses with the highest dose in the evening.

A long-term follow-up of the same patients showed that the average oral dDAVP dose was similar (906 ± 406 µg/24 h) after 1 to 3.5 years of treatment (Fjellestad-Paulsen *et al.*, 1993a). No adverse reactions related to oral dDAVP and no presence of circulating antibodies against dDAVP were observed.

In these studies the oral dose of dDAVP could not be correlated to age, to body weight or to the severity of the pre-treatment polyuria and the same was found in the 10 pediatric DI patients in study VI presented in Table 2. This is in accordance with previous findings with intranasal dDAVP (Kauli *et al.*, 1985).

In two studies in adult DI patients (Hammer and Vilhardt, 1985; Cunnah *et al.*, 1986) the daily oral dDAVP was lower than in our studies (Table 8). Heterogenous patient selection could be one reason and the low number of patients in each study (7 - 10 patients). Pharmacokinetics of dDAVP may differ between children and adults, a higher water turnover is more often tolerated in adults and as children need more sleep at night, their evening doses are increased in comparison to adults. The mean daily dDAVP dose in our studies is however only slightly above that found in a study with 7 children with central DI aged 4 - 18 years (Westgren *et al.*, 1986).

In a pharmacokinetic study in overhydrated normal subjects and in adults and children with central DI it was observed that the children were less sensitive than normal subjects to a 50 µg dose and required 100 µg orally to achieve a duration of action similar to that resulting from 200 µg in adults (Williams *et al.*, 1986).

In order to determine the relationship between the intranasal and oral doses of dDAVP a pharmacokinetic study (VI) was undertaken in 10 DI patients aged 4.5 to 19 years (Table 7). Comparing the AUC after 10 and 20 µg given intranasally and after 200 and 400 µg given

orally, a ratio of 1:20 was found between the intranasal and oral routes. When comparing the oral dDAVP, in a dose ranging study in seven pediatric patients with hypothalamic DI, with the previous intranasal daily dosage a similar correlation was found (Westgren *et al.*, 1986). In previous studies, where plasma dDAVP was measured by means of an anti-serum non-specific for dDAVP, the plasma half-life ranged between 51 min (Edwards *et al.*, 1973) and 158 min (Pullan *et al.*, 1978) after i.v. administration and varied from 0.4 to 4 h after i.n. administration (Seif *et al.*, 1978).

In these studies there was no correlation between elimination of dDAVP from plasma and duration of antidiuretic effect suggesting that the inter-individual differences observed in duration are related to the absorption.

Using a specific RIA, Vilhardt *et al.* (1986) found a plasma half-life of 55 min after i.v. administration in healthy volunteers. We found a plasma half-life of 158 to 238 min after i.n. and oral routes of administration without significant difference between the two routes.

In study VI the peak plasma concentrations of dDAVP appeared early and plasma levels were still detectable after 8 h indicating a rapid transmucosal absorption from the gastrointestinal tract. The persistence of dDAVP in the plasma after oral administration is probably due to slow elimination of the drug or to a prolonged absorption phase of the peptide as the half-life after i.v. administration is much shorter.

The longer half-life after intranasal and oral administration of dDAVP is probably responsible for the increased duration of antidiuresis in comparison to vasopressin whose half-life is approximately 5 minutes.

In uraemic patients Aunsholt *et al.* (1986) found that the total clearance rate was lower (1.4 ml/min/kg) than previously reported in healthy volunteers (2.2 ml/min/kg) and the half-life of dDAVP in plasma was much longer 200 min (Table 7). This decreased rate of elimination in uraemic patients may be caused by diminished renal excretion and may account for the prolonged effect on bleeding and prolonged increase of factor VIII (Mannucci *et al.*, 1983).

The treatment of neonatal DI is particularly difficult. The intranasal administration of dDAVP does not seem suitable in infants because they cannot inhale and irregular nasal absorption can be responsible for frequent episodes of hypernatremia and hyponatremia. These episodes cease when the children are treated orally (Fjellestad-Paulsen and Czernichow, 1992). The dose requirements are very low at this age but a ratio of 1:10 between the intranasal and oral routes indicate a higher bioavailability before the age of 6 months (Fjellestad-Paulsen *et al.*, 1993b).

It is well known that a considerable passage of macromolecules occurs through the intestinal wall during the perinatal period in many species (Brambell, 1970; Vellenga *et al.*, 1985; Baintner, 1986). After a short period this passage is reduced and a so-called closure

phenomenon or "macromolecular closure" occurs when the intestine matures (Walker *et al.*, 1972; Rodewald, 1973). In 14-day-old rats the transepithelial intestinal passage of dDAVP was higher than in 30-day-old rats (Folkesson *et al.*, 1988).

Preterm but also full-term infants can absorb a considerable amount of intact protein across the gastrointestinal wall. It is a progressive event starting before birth and continuing until at least the age of 5 months (Gardner, 1994) thereafter decreasing progressively.

To summarize the treatment of diabetes insipidus: despite very low bioavailability, oral dDAVP induces a rapid, potent and sustained antidiuresis in diabetes insipidus patients and the treatment with oral dDAVP is an attractive alternative to the intranasal route of administration. Approximately twenty to thirty times larger doses are needed orally than intranasally, but this does not result in an increase in side effects. The recommended dosage of oral dDAVP for children and adults with central DI would be 100 to 300 μ g two or three times daily. The recommended dose in infants, before the age of 6 months, would be 5 - 10 μ g two to three times daily, which is lower than previously indicated (Fjellestad-Paulsen *et al.*, 1988).

Future approaches to improve the oral absorption of neurohypophyseal hormones

Oral absorption of dDAVP has been facilitated by molecular modifications of the parent molecule which have rendered the molecule more resistant to proteolysis than native AVP. Further molecular changes, having a direct action or not on the opening of tight junctions (Yen and Lee, 1995) or a lipophilic pro-drug form (Kahns *et al.*, 1993) might increase resistance to chymotrypsin but the intact antidiuretic effect of the molecule must be conserved. Addition of an enzyme inhibitor increases the bioavailability but there are concerns about potential side effects. Use of absorption enhancers that could increase the passive diffusion by increasing the permeability of tight junctions is an attractive alternative but local toxicity with epithelial damage and increased transmucosal transport of undesired substances are possible drawbacks. Peptide carriers in nanocapsules or hydrophobic formulations in micro-emulsions are additional alternatives (Demgé, 1988; Muranashi, 1990; Ritschel, 1991).

CONCLUSIONS

The different studies (I - VI) have further characterized the gastrointestinal fate of neurohypophyseal hormones and their analogues, especially of dDAVP, and the following conclusions can be drawn:

- ◆ The major enzymatic barriers to intestinal absorption of AVP, oxytocin and their analogues are present in the intestinal contents and not in the mucosa, which however constitutes a major physical barrier to peptide transport.
- ◆ Degradation of AVP, oxytocin and antocin is rapid when incubated with renal microvilli membranes together with reduced glutathione while dDAVP is stable.
- ◆ Oxytocin, AVP and dDAVP are metabolized when incubated in crude liver homogenates indicating that the proteolytic activity takes place intracellularly rather than in the plasma membrane.
- ◆ In the present study the bioavailability after intranasal and oral administration of dDAVP was found to be in the order of 3% and 0.1% respectively. The bioavailability after oral administration seems lower than previously reported. This bioavailability ratio of approximately 1:30 is confirmed by clinical experience when patients change from intranasal to oral dosing.
- ◆ The bioavailability of dDAVP after direct application in the stomach, duodenum and the jejunum is similar (0.2%) to that after swallowing a tablet and is significantly lower after direct application in the ileum, colon and rectum (0.04%). Absorption of dDAVP from the distal part of the ileum is lower than expected from animal studies and no preferential site of absorption is found within the upper segment of the gastrointestinal tract.
- ◆ The bioavailability of dDAVP after direct duodenal application increases five times with a simultaneous intestinal perfusion of an enzyme inhibitor demonstrating the importance of the proteolytic effect of intestinal enzymes on the intestinal absorption of dDAVP.
- ◆ Despite very low bioavailability, oral dDAVP induces a rapid, potent and sustained antidiuresis in paediatric diabetes insipidus patients and the treatment with oral dDAVP is an attractive alternative to the intranasal route of administration. Approximately twenty to thirty times larger doses are needed orally than intranasally, but this does not result in an increase in side effects.

SUMMARY

Although gastrointestinal absorption of intact and biologically active peptides has been extensively studied in the last two decades there is sparse information, especially in man, on the gastrointestinal hydrolysis and disposition of such biologically active peptides as the neurohypophyseal hormones and their analogues. The general aim of this investigation was to study the absorption and metabolism of these hormone peptides, especially 1-deamino-8-D-arginine vasopressin (dDAVP), in human tissue, in healthy volunteers and in patients with hypothalamic diabetes insipidus (DI).

Central or hypothalamic DI, a chronic state of polyuria and polydipsia, is due to absent or impaired production or secretion of vasopressin (AVP) secondary to different pathologies in the hypothalamo-pituitary region.

The treatment of DI is based on substitution-therapy of AVP and since 30 years on a synthetic analogue of AVP, dDAVP, a potent antidiuretic with prolonged activity usually administered intranasally (i.n.). Previously it was generally believed that peptides could not cross the gastrointestinal mucosal barrier without being hydrolysed to free amino acids. However it has been shown that dDAVP given orally retains its biological activity and must therefore be absorbed in an intact form.

In study VI we demonstrated that oral treatment with dDAVP in children with DI is possible and effective in comparison to the i.n. form. The bioavailability is, however, very low and approximately twenty to thirty times larger doses are needed orally than intranasally, but without resulting in an increase in side effects.

The bioavailability of dDAVP was studied in a pharmacokinetic study in healthy volunteers (III) after intravenous, subcutaneous, i.n., oral, sublingual and rectal administration. Half-life of elimination was 78 ± 10 min. The bioavailability was 3.4% and 0.1% after i.n. and oral administration, respectively. Absorption after sublingual and rectal administration was almost negligible. The bioavailability after oral administration was lower than previously reported.

In study IV the bioavailability was studied in healthy volunteers after dDAVP was given by an intestinal perfusion technique to 6 different sites of the gastrointestinal tract. The bioavailability after administration in the stomach, the duodenum and the jejunum was similar to that after swallowing a tablet (study III) and was significantly higher than after direct application in the ileum, colon and rectum. No preferential site of absorption was found within the upper segment of the gastrointestinal tract.

In study I the metabolism of AVP, oxytocin (OT) and some of their analogues was studied in undiluted human intestinal contents and purified microvilli membranes from different parts of the intestine. When incubated in intestinal contents AVP was completely metabolized within 30 min whereas an extensive but slower and pH-dependent degradation of dDAVP occurred, especially in the ileum. This latter was inhibited in a concentration-dependent manner when the enzyme inhibitor aprotinin was preincubated with contents from the ileum. Among the uterotonic peptides, C-terminal amides seemed to be more stable than acids. Deamination at position 1 and substitution of L-Tyr with D-Tyr(Et) at position 2 in the ring structure of the oxytocin molecule increased resistance to chymotrypsin.

When incubated with gastrointestinal brush-border microvilli membranes from the ileum, AVP and OT were only metabolized after 180 min and in the presence of reduced glutathione. Synthetic analogues were stable under identical conditions. The major enzymatic barriers to intestinal absorption of AVP, oxytocin and their analogues are present in the intestinal contents and not in the mucosa, which however constitutes a major physical barrier to peptide transport. In study II when AVP, OT and their analogues were incubated for 180 min with human renal microvilli membranes dDAVP was stable while AVP, OT and [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-OT followed different metabolic pathways and were rapidly metabolized in the presence of glutathione. All peptides except antocin were degraded after 180 min when incubated with crude liver homogenate. This did not occur with purified plasma membranes indicating that the proteolytic activity takes place intracellularly rather than in the membranes.

Finally, in study V the bioavailability of dDAVP after direct duodenal application increased 5 times through a simultaneous perfusion of an enzyme inhibitor, demonstrating the effect of proteolytic intestinal enzymes on the absorption of dDAVP.

RÉSUMÉ (Summary in French)

L'absorption et le métabolisme des hormones posthypophysaires (la vasopressine et l'oxytocine) et de leurs analogues, particulièrement la l-deamino-8-D-arginine vasopressine (dDAVP) ont été étudiés dans différents tissus humains, chez des volontaires sains et chez des enfants atteints de diabète insipide hypothalamique.

Le diabète insipide (DI) central ou hypothalamique, caractérisé par un syndrome polyuro-polydipsique chronique, est dû à l'absence de production ou de libération de vasopressine (AVP), ou hormone antidiurétique (ADH), elle-même secondaire à une atteinte de l'axe hypothalamo-hypophysaire.

Le traitement du DI central repose sur la substitution en VP, ou surtout depuis 30 ans, sur un analogue de la VP, la dDAVP, antidiurétique puissant à action prolongée, administrée par voie nasale. Cette molécule synthétique a permis une potentialisation de l'effet antidiurétique parallèlement à une réduction quasi complète de l'activité pressive et utéro-tonique.

Alors qu'il était généralement accepté, que les peptides ne pouvaient pas passer la barrière gastro-intestinale sans être hydrolysés en acides aminés libres, il a été démontré que même administrée par voie orale, la dDAVP garde son activité biologique. Elle est en effet absorbée dans sa forme intacte.

Nous avons pu montrer dans une étude (VI) comparative chez des enfants atteints de diabète insipide central avec la forme intranasale (i.n.), que la forme orale de dDAVP était efficace. Cependant, du fait d'une biodisponibilité très faible, des doses 20 à 30 fois plus fortes étaient nécessaires par voie orale par rapport à la voie nasale sans toutefois engendrer des effets secondaires.

La biodisponibilité de la dDAVP a été étudiée chez le volontaire sain, dans une étude pharmacocinétique (étude III), en comparant les dosages de la dDAVP plasmatique après administration intraveineuse, sous-cutanée, i.n., orale, sublinguale et rectale. La biodisponibilité de la dDAVP après administration i.n. et orale a été évaluée à 3.4% et 0.1% respectivement. La biodisponibilité par voie orale était plus faible que précédemment décrite. Les taux plasmatiques de la dDAVP ne s'élevaient pas après administration sublinguale ou rectale, mais de faibles concentrations étaient retrouvées dans les urines des 24 heures. La dégradation des hormones posthypophysaires et analogues a été étudiée lors d'incubation dans le suc gastrique et intestinal et en présence de micro-villosités de la bordure en brosse de l'intestin grêle ou de membranes plasmiques de l'estomac, du rectum et du colon (étude I).

L'AVP a été métabolisé très rapidement dans le suc intestinal non dilué au niveau de la partie distale de l'iléon. La dégradation de la dDAVP a été importante, mais plus lente et pH-dépendante surtout, au niveau de l'iléon. Cette dernière a été inhibée par l'adjonction d'un inhibiteur enzymatique, l'aprotinine. Parmi les peptides utérotoniques testés, les formes amides étaient plus stables que les formes acides. La molécule d'oxytocine déaminée en position 1 possédant de plus un L-Tyr substitué par un D-Tyr (Et) en position 2 dans la structure cyclique, s'est montrée résistante envers la chymotrypsine.

Lors de l'incubation dans les membranes micro-villositaires, l'AVP et l'oxytocine n'ont été métabolisés qu'après 180 minutes et qu'en présence de glutathion réduit, alors que les analogues synthétiques ont été stables dans les mêmes conditions. Ainsi, la dégradation enzymatique de l'AVP, de l'oxytocine et de leurs analogues se situe essentiellement dans le suc intestinal et non au niveau de la membrane épithéliale qui constitue cependant une barrière physique importante au transport des peptides.

Lors de l'incubation dans des membranes micro-villositaires rénales humaines (étude II), la dDAVP était stable, alors que l'AVP, l'oxytocine et la [Mpa¹-DTyr², Thr⁴, Orn⁸]-oxytocine (antocine) étaient dégradés de façon différente et rapidement métabolisés en présence de glutathion réduit. Aucune dégradation peptidique n'a été observée lors de l'incubation dans des membranes purifiées hépatiques, alors que tous les peptides testés, sauf l'antocine, étaient dégradés lors de l'incubation dans des homogénats cellulaires. La dégradation hépatique paraît ainsi intracellulaire plutôt que membranaire.

Finalement, dans l'étude V, la biodisponibilité de la dDAVP administrée avec ou sans aprotinine dans le duodenum a été étudiée chez des volontaires sains. La biodisponibilité de la dDAVP, après administration directe duodénale, était similaire à celle obtenue après administration de comprimés (étude III) et elle était augmentée cinq fois lors de la co-administration d'un inhibiteur enzymatique.

FERING TUPFAADING (Summary in Frisian)

Hû a hormoone faan a bääftlaap faan a branjknoop an høg analooge fööraal l-deamino-8-D-arginine-vasopressin (dDAVP), uun humaan weew, bi sünj mensken an bi patienten mä hypothalamisk diabetes insipidus apnimen an deembreegen word, as onersooght.

AVP, wat uun unfertanet tiareminholt inkubiiret wiär, wiär uun 30 minuuten metabolisiiret, widjert dDAVP extensiver, man uk suutjiser uun ferskelig dialen faan a tiarem, fööraal uun't ileum deembreegen word. At deembreegen word hemet, wan a ileuminholt iarst mä a enzyhmemer Aprotinin inkubiiret wiär. Da hir efekt wiär ufhingig faan a koncentration. Mad a uterotonsk peptiden san a C-terminal peptiden skiinboor muar stabiil üüs a söringen. Deamination uun position 1 an substitution faan L-Tyr mä D-tyr (Et) uun position 2 faan oxytocins ringstruktuer ferhuugri a resistens jin chymotrypsin.

Wan a natürelk peptiden AVP an oxytocin uun an homogenaat faan gastrointestinaal mikrovilli-membraanen faan a distal dial faan at ileum inkubiiret word, saan jo ban 180 minuuten deembreegen, uk wan reduciiret glutathion diarbi as. Synteetisk analoogen (dDAVP, Antocin, Carbetocin) wiär oner likedening bedingen stabiil. Uun an mucosahomogenaat faan a maag küd ham nian deembreegen sä, widjert AVP uun't jejunum, uun at colon an at rektum suutjis deembreegen word.

Wan vasopressin, oxytocin an jar analoogen 180 minuuten loong mä mikrovillimembraanen faan at lenlaag inkubiiret word, wiär dDAVP stabiil, widjert AVP, oxytocin an [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-oxytocin rask deembreegen word, wan glutathion diarbi wiär. Al peptiden, bütj Antocin, wiär ban 180 minuuten deembreegen, wan jo mä rä liwerhomogenaat inkubiiret wiär, dDAVP extensiver üüs AVP. Det skäet ej mä rensket plasmamembraanen. A proteolytisk aktivitet ferlept diaram iarer intracelulär üüs uun a membraanen.

Efter intranasal administration faan dDAVP wiär 3%, an efter oral administration 0.1% biologisk disponiibel. A biodisponibilitet faan dDAVP wiär efter direkt aplikation uun maag, duodenum an jejunum (0.2%) lik den efter ian tablet an signifikant huugher üüs efter direkt aplikation uun't ileum, colon an rektum (0.04%). Nian faan a boowenst segmenten faan a tiarem häd an föörtöoch faan a absorption. A biodisponibilitet faan dDAVP wiär efter direkt duodenal aplikation bi perfusion mä an enzyminhibitor 5sis so grat, wat a bidüüding faan a proteolytisk tiaremenzymen för absorption faan dDAVP wiset. Uk wan a biodisponibilitet faan oral dDAVP liag as, so feert at bi pediatrik patienten mä diabetes insipidus tu an rask, deeg antidiures faan loong düür. A terapii mä oral dDAVP as an atraktiiv alternatiiv tu a intranasal administration. A dosiiring as oral 20 - 30sis so huugh üüs intranasal, wat dach ej tu muar biwirkingen feert.

DEUTSCHE ZUSAMMENFASSUNG (Summary in German)

Die Absorption und der Abbau der Hypophysenhinterlappenhormone, und einiger Analoge, insbesondere dDAVP wurde in humanem Gewebe, bei gesunden Menschen und bei Patienten mit hypothalamischem Diabetes insipidus untersucht.

AVP, das in unverdünntem Darminhalt inkubiert wurde, war innerhalb von 30 Minuten metabolisiert, während dDAVP extensiver, aber langsamer, in verschiedenen Teilen des Darms, insbesondere im Ileum, abgebaut wurde. Dieser Abbau wurde in Konzentrationsabhängiger Weise gehemmt, wenn der Enzymhemmer Aprotinin mit Ileuminhalt vorher inkubiert wurde. Unter den uterotonischen Peptiden scheinen C-terminale Peptide stabiler zu sein als Säuren. Deamination in Position 1 und Substitution von L-Tyr durch D-Tyr(Et) in Position 2 der Ringstruktur des Oxytocins erhöhen die Resistenz gegen Chymotrypsin.

Wenn die natürlichen Peptide AVP und OT im Homogenisat gastrointestinaler Mikrovill-Membranen vom distalen Teil des Ileums inkubiert wurden, wurden sie innerhalb von 180 Minuten, auch in Gegenwart von reduziertem Glutathion, abgebaut. Synthetische Analoge (dDAVP, Antocin, Carbatocin) waren unter gleichartigen Bedingungen stabil. Im Mucosahomogenat vom Magen wurde kein Abbau gesehen, während ein langsamer Abbau von AVP im jejunum, im Colon und im Rektum geschah.

Wenn Vasopressin, Oxytocin und ihre Analoge 180 Minuten lang mit humanen Mikrovillmembranen der Niere inkubiert wurden, war dDAVP stabil, während AVP, Oxytocin und [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-Oxytocin in Gegenwart von Glutathion schnell abgebaut wurden. Alle Peptide, ausser Antocin, waren innerhalb von 180 Minuten abgebaut, wenn sie mit rohem Leberhomogenat inkubiert wurden, dDAVP extensiver als AVP. Dies geschah nicht mit gereinigten Plasma-membranen. Die proteolytische Aktivität fand also eher intracellulär statt, als in den Membranen.

Nach intranasaler und oraler Administration von dDAVP wurden 3% bezwecks 0.1% als biologisch verfügbar gefunden. Die Bioverfügbarkeit von dDAVP war nach direkter Applikation in Magen, Duodenum und Jejunum (0.2%) ähnlich der nach Einnahme einer Tablette und signifikant höher als nach direkter Applikation in Ileum, Colon und Rektum (0.04%). Keines der oberen Segmente des Darms zeigte eine besondere Absorption. Die Bioverfügbarkeit von dDAVP nach direkter duodenaler Applikation war bei gleichzeitiger Perfusion eines Enzyminhibitors 5mal grösser, was die Bedeutung proteolytischer Darmenzyme für die Absorption von dDAVP beweist. Trotz der sehr niedrigen Bioverfügbarkeit, führt orales dDAVP zu einer schnellen, kräftigen und andauernden Antidiurese bei pädiatrischen Patienten mit Diabetes insipidus. Die Behandlung mit oralem dDAVP ist eine attraktive Alternative zur intranasalen Administration. Oral sind etwa 20 - 30mal höhere Dosen notwendig als intranasal, was aber nicht zu einer Steigerung der Nebenwirkungen führt.

SVENSK SAMMANFATTNING (Summary in Swedish)

Absorption och metabolism av baklobshormonerna arginin-vasopressin (AVP), oxytocin och deras syntetiska analoger, i synnerhet 1-deamino-8-D-arginin vasopressin (dDAVP) har studerats i olika vävnader från människa, hos friska frivilliga samt hos barn med hypothalamisk diabetes insipidus.

Central eller hypothalamisk diabetes insipidus (DI), karakteriseras av ett kroniskt polyuri-polydipsi syndrom (ökad urinmängd och törst) och beror på sänkt produktion eller frisättning av vasopressin, som i sin tur beror på olika patologiska tillstånd i hypothalamo-hypofysära regionen.

Behandling av central DI bygger på substitutionsterapi med vasopressin, alternativt en analog av vasopressin nämligen dDAVP, ett antidiuretikum med kraftfull och förlängd effekt som normalt tillförs intranasalt (i.n.). Denna syntetiska molekyl har fört med sig en förstärkning av den antidiuretiska effekten parallellt med en nästan upphävd vasopressiv och uterotonisk effekt.

Tidigare var det allmänt vedertaget att peptider inte kunde gå över mag- och tarmkanalbarriären utan att brytas ner till fria aminosyror. Det har emellertid påvisats att dDAVP även efter oral tillförsel behåller sin biologiska effekt dvs är upptagen i intakt form.

Behandling av barn med DI med oralt dDAVP var lika effektiv som med den intranasala formen (delarbete VI). Biotillgängligheten var dock mycket låg och man behövde orala doser som var 20 till 30 gånger större än de intranasala vilket dock inte ökar biverkningarna.

Biotillgängligheten av dDAVP studerades i en farmakokinetisk studie hos friska frivilliga (delarbete III) efter intravenös (i.v.), subkutan, i.n., oral, sublingual och rektal tillförsel. Biologiska effekter mättes och plasma- och urinkoncentrationer av dDAVP bestämdes med en specifik RIA-metod. En signifikant ökning av urinosmolaliteten påvisades vid olika tillförselvägar, under upptill 8 timmar efter administrering, fränsett efter sublingual och rektal tillförsel. Halveringstiden efter i.v. tillförsel var 78 ± 10 min. Biotillgängligheten av dDAVP var 3.4% efter intranasal tillförsel, och 0.1% efter oral tillförsel. Inga mätbara plasmakoncentrationer noterades efter sublingual och rektal tillförsel, men låga koncentrationer påvisades i 24-timmarsurin. Man kan dra slutsatsen att dDAVP's biotillgänglighet är lägre efter oral tillförsel än som tidigare rapporterats.

I delarbete IV har biotillgängligheten av dDAVP undersökts hos sex frivilliga. dDAVP tillfördes via sond till sex skilda avsnitt av gastrointestinalkanalen (ventrikel, duodenum, jejunum, ileum, kolon, rektum). Biotillgängligheten av dDAVP efter tillförsel till ventrikeln, duodenum och jejunum motsvarade vad som tidigare påvisats efter tillförsel i tablettform (delarbete III). Absorptionen från ileum var lägre än förväntat, och inget predilektionsställe för absorption kunde påvisas.

I delarbete I har nedbrytning av AVP, oxytocin (OT) och några av deras syntetiska analoger studerats efter inkubation i ventrikelsekret, tarmsekret och vävnadshomogenisat från olika avsnitt av gastrointestinalkanalerna. En snabb nedbrytning av AVP påvisades i ospätt sekret från ileum medan en betydande men långsammare och pH-beroende nedbrytning skedde av dDAVP. En koncentrationsberoende hämning påvisades efter tillsats av protininhibitorn aprotinin. När peptiderna inkuberades i homogenisat från microvillimembran i ileum bröts AVP och oxytocin först ner efter 180 min och endast i närvaro av reducerat glutation. Den huvudsakliga enzymatiska barriären mot intestinal absorption av vasopressin, OT och deras analoger finns alltså i tarmsekret och inte i tarmmucosan som dock är ett betydande fysikaliskt hinder för absorption.

I delarbete II har metabolismen av OT, AVP och analogerna dDAVP och antocin studerats i homogenisat från humana renala mikrovillimembran, och levermembran. dDAVP var stabilt i renala mikrovillimembran medan AVP, OT och [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-oxytocin uppvisade olika nedbrytningsvägar och bröts snabbt ned i närvaro av reducerat glutation. Ingen nedbrytning sågs när peptiderna inkuberades med homogeniserade humana levermembran, medan samtliga peptider utom antocin metaboliserades vid inkubation med orenad, homogeniserad levervävnad. Detta talar för att nedbrytningen i levern snarare är intracellulär än membranbunden.

I delarbete V har slutligen biotillgängligheten av dDAVP tillfört med och utan enzyminhibitorn aprotinin via duodenalsond till friska frivilliga studerats. Undersökningen visade att biotillgängligheten av dDAVP efter direkt duodenal tillförsel av dDAVP motsvarar vad som tidigare påvisats efter tillförsel av tabletter, men biotillgängligheten ökar 5 gånger vid samtidig tillförsel av enzyminhibitorn aprotinin.

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Metabolism of Vasopressin, Oxytocin, and Their Analogues in the Human Gastrointestinal Tract

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FJELLESTAD-PAULSEN, A., C. SÖDERBERG-AHLM AND S. LUNDIN. *Metabolism of vasopressin, oxytocin, and their analogues in the human gastrointestinal tract*. PEPTIDES 16(6) 1141–1147, 1995.—The bioavailability from the gastrointestinal tract of peptides as large as nonapeptides is very low, which may be attributed to extensive luminal and mucosal degradation. The aim of the present study was to investigate the stability of the neurohypophyseal hormones arginine-vasopressin (AVP), oxytocin (OT), and their synthetic analogues in human intestinal contents, small intestinal brush-border membranes, and gastric, rectal, and colonic plasma membranes. Peptides were incubated in gastrointestinal contents from healthy volunteers and in human intestinal mucosa homogenates. The extent of degradation was determined by reversed-phase high performance liquid chromatography (HPLC). AVP was rapidly degraded in the ileum fractions of the intestinal contents whereas 50% of the analogue 1-deamino-8-D-arginine vasopressin (dDAVP) remained intact after 35 min. The degradation was pH dependent, and a concentration-dependent inhibition was observed when aprotinin, a proteinase inhibitor, was preincubated with contents from the ileum. No degradation of AVP, dDAVP, or oxytocin analogues was observed in the mucosa homogenate from the stomach. The peptides were found to be rather slowly degraded by intestinal microvilli membranes and colonic and rectal plasma membranes. This degradation occurred essentially when reduced glutathione 10^{-4} M was added to the incubations. In conclusion, the major enzymatic barrier to intestinal absorption of OT, VP, and their analogues is present in the intestinal juice and not in the mucosa, which, however, constitutes a major physical barrier to peptide transport.

Arginine-vasopressin Brush-border membranes Gastric juice Gastrointestinal tract Human Metabolism
 Oxytocin

THERE is scant information on the stability and absorption of biologically active peptides in the gastrointestinal (GI) tract. It was demonstrated by Matthews et al. in 1969 in animals (16), and later in man (1), that di- and tripeptides could undergo intestinal absorption by active mechanisms. The nonapeptide 1-deamino-8-D-arginine-vasopressin (dDAVP), used successfully intranasally in patients with central diabetes insipidus (DI) (2), was later shown to elicit antidiuresis after oral administration in conscious dogs (20), in normal healthy volunteers (21), and in diabetes insipidus patients (10). Buccal administration of oxytocin (OT) to pregnant women at term is followed by uterine contractions and increased plasma concentrations of oxytocin (6).

The fact that bioactive peptides as large as nonapeptides can be absorbed by the GI tract is attractive from a therapeutic point of view. However, we know that the bioavailability of these peptides is very low and therefore this route of delivery is only feasible for highly potent substances. In a pharmacokinetic study comparing the intranasal and the oral routes of dDAVP administration to children with DI a ratio of 1:20 was found between the two routes of administration (7). A pharmacokinetic study in healthy volunteers showed an oral bioavailability of 0.1% of

dDAVP (8) and the absolute bioavailability of an aqueous solution of dDAVP administered in the jejunum was 0.2% (5). The metabolism of vasopressin and oxytocin in the GI tract in animals is not fully elucidated and in man it is more or less unknown. Improved stability of dDAVP compared to arginine-vasopressin (AVP) was shown in intestinal contents of the rabbit (17) and in pancreatic juice of the pig (13).

The present study was undertaken to study the stability of the neurohypophyseal hormones (AVP and OT) as well as some of their synthetic analogues in human intestinal contents and brush-border membranes from the GI tract. Information gained from such studies is essential for the development of degradation-resistant peptides for oral delivery.

METHOD

Peptides and Chemicals

The molecular structures and abbreviations of the investigated peptides are shown in Table 1. All unlabeled peptides were synthesized by solid-phase method (Ferring Pharmaceuticals, Malmö, Sweden) and had a chromatographic purity of > 98%. A large spectrum proteinase inhibitor, aprotinin, was obtained

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TABLE 1
UTEROTONIC PEPTIDES AND VASOPRESSIN ANALOGUES
USED IN THE PRESENT STUDY

| Peptide | Amino Acid Sequence |
|------------------------------|--|
| Uterotonic Peptides | |
| Antocin | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Cys-Pro-Orn-Gly-NH ₂ |
| CAP-OH | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Cys-Pro-Orn-Gly-OH |
| 8-DArg-CAP | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Cys-Pro-DArg-Gly-NH ₂ |
| desGly-CAP | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Cys-Pro-Orn-NH ₂ |
| desGly-NH ₂ -CAP | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Cys-Pro-Orn-OH |
| 6-Carba-desGly-CAP | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Abu-Pro-Orn-NH ₂ |
| Carbetocin | Bua-Tyr(Me)-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH ₂ |
| Oxytocin | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH ₂ |
| Vasopressin Analogues | |
| AVP | Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH ₂ |
| dDAVP | Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-Gly-NH ₂ |
| desGly-dDAVP | Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-NH ₂ |

Bua = butyric acid, Mpa = mercapto propionic acid.

from Boehringer Mannheim. Glutathione (reduced form) and a specific chymotrypsin inhibitor (chymostatin) were purchased from Sigma.

Human Gastrointestinal Juice

Preparation of juice and intestinal contents. Four healthy male volunteers, aged 20–32 years, participated in the study after giving written informed consent. The study was approved by the Hospital Ethics Committee. Each volunteer came to the investigation unit at 1600 h after a 8-h fasting period. A tube was introduced intranasally after local anaesthesia with xylocaine and the correct location of the tube was ascertained by fluoroscopic control. Gastric juice was collected from the fasting participants the same afternoon. Thereafter, one standardized meal was given 1 h before sampling of duodenal and distal jejunal contents and another the next morning 2 h before the distal ileal contents collection. After centrifugation the gastric juice and intestinal contents were frozen in aliquots of 1 ml and stored at -20°C .

Degradation in gastric juice or intestinal contents. The degradation of peptides by gastric juice or intestinal contents was assessed by adding 10 μl 10 mM peptide in 0.9% NaCl to 190 μl undiluted juice. The reaction was allowed to proceed at 37°C . Aliquots (25 μl) were withdrawn at intervals and mixed with 100 μl acetone to stop the reaction. After centrifugation for 10 min at $10,000 \times g$, 10 μl of the supernatant was analyzed by reversed-phase high performance liquid chromatography (HPLC). The effects of increasing amounts of enzyme inhibitors were examined under reaction conditions in intestinal contents from the ileum.

Determination of peptide degradation. The extracted samples were analyzed using an HPLC system consisting of a Varian 5000 apparatus equipped with a Varian UV-100 detector, Rheodyne Model 7125 injector, and Varian 4290 integrator. Mobile phases were filtered through 0.45- μm filters (Schleicher & Schuell). The peptides were chromatographed isocratically in a system of MeOH/0.025 M ammonium acetate on a μ -BondapakTM C18 (3.9 \times 300 mm) chromatographic column and a μ -BondapakTM C18 guard column. The flow rate was 1 ml/min and UV absorbance was determined at 220 nm.

pH and protein determination. The pH measurements were performed with Orion pH meter, model SA720, and pH paper

(Merck), special indicator pH 4.0–7.0. Protein determinations of gastrointestinal juice were made with Bio-Rad protein assay.

Brush-Border and Plasma Membranes

Tissue sampling. Small fractions (10 \times 30 mm) of intestinal epithelium from various parts of the gastrointestinal tract were collected in patients undergoing surgery for localized tumors. Tissue was obtained from the stomach, the proximal segment of the jejunum and the distal part of the ileum, from the proximal segment of the colon, and from the rectum, and was stored at -70°C until required.

Preparation of membranes. Human intestinal microvilli brush-border membranes were prepared according to the method described by Booth and Kenny (3). Briefly, the frozen gastric or intestinal tissue specimens were thawed at room temperature and the mucosal layer was then scraped off and immersed in ice-cold isotonic saline. Tissue (1 cm^2) from jejunum and ileum was homogenized (Polytron model PT 10/35 Kinematica) for 3 \times 9 s in 20 ml ice-cold Mannitol (10 mM) Tris-HCl (2 mM, pH 7.10, 4°C). Solid $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was added to give a concentration of 10 mM and the homogenate was then stirred occasionally for 15 min in an ice bath. The homogenate was thereafter centrifuged (1500 $\times g$, 15 min, 4°C). From the jejunum and the ileum two pellets, respectively, were resuspended in 20 ml buffer (Mannitol-Tris- MgCl_2) and were homogenized and centrifuged (2200 $\times g$, 15 min, 4°C). Then the supernatant was centrifuged (15,000 $\times g$, 15 min, 4°C) and the final pellet from each centrifugation was resuspended in 5 ml buffer and briefly homogenized. Membranes were stored in aliquots at -70°C . Protein content of the membranes was measured according to Lowry et al. (12). The purity of the final membrane pellet was determined by measurements of membrane markers such as alkaline phosphatase (9) and a lysosomal marker (cathepsin B).

Tissue from the stomach and the colon was homogenized as described above. The homogenate was centrifuged (2000 $\times g$, 5 min, 4°C), the supernatant was ultracentrifuged (Beckman, 100,000 $\times g$, 45 min, 4°C). The pellet was then briefly homogenized in buffer containing 0.5 M NaCl and ultracentrifuged as above. The final pellet was resuspended in 4 ml buffer and aliquots were stored at -70°C . Tissue from the rectum was homogenized as previously described and thereafter centrifuged twice for 2 h (48,000 $\times g$, 4°C).

TABLE 2
PROTEIN CONTENT AND pH VALUES OF UNDILUTED GASTRIC JUICE AND
INTESTINAL CONTENTS FROM FOUR HEALTHY VOLUNTEERS

| | Person 1 | Person 2 | Person 3 | Person 4 |
|--------------------------------|----------|----------|----------|----------|
| Protein concentrations (mg/ml) | | | | |
| Gastric juice | 0.139 | 0.312 | 0.055 | 0.271 |
| Duodenum | 0.573 | 0.804 | 0.732 | 0.342 |
| Jejunum | 1.392 | 1.152 | 1.270 | 0.651 |
| Ileum | 0.736* | 1.662 | 1.878 | 1.548 |
| pH values | | | | |
| Gastric juice | 3.5 | 6.5† | 1.5 | 2.0 |
| Duodenum | 3.0 | 5.0 | 4.8 | 4.0 |
| Jejunum | 5.3 | 5.3 | 5.3 | 5.3 |
| Ileum | 4.7* | 6.2 | 6.2 | 6.2 |

* This sample was probably taken from the lower part of the jejunum.

† There were technical difficulties in obtaining gastric juice from the patient.

Degradation in human intestinal brush-border and plasma membranes. AVP, dDAVP, oxytocin, carbetocin, and [Mpa¹,D-Tyr²(Et),Thr³]oxytocin (Table 1) were dissolved in a medium consisting of 100 mM Tris-HCl containing 150 mM NaCl at pH 7.4. The incubation mixture consisted of 1–10 µg membrane protein and 10⁻⁴ M of peptide in a final volume of 100 µl. To half of the incubations, a cofactor (glutathione 10⁻⁴ M) was added, which is removed in the microvilli membrane purification step (16). The incubations, in duplicate, proceeded at 37°C in a rotating water bath and after 0, 30, and 180 min the reaction was stopped by boiling for 5 min followed by centrifugation.

Determination of peptide degradation. Peptides were identified by HPLC employing a KONTRON HPLC pump 420 equipped with an autosampler, and UV detector 430 and a KONTRON 460 integrator. Mobile phases were filtered through 0.45-µm filters (Schleicher & Schuell).

The column (µBondapak™ C18, 300 × 3.9 mm, Waters Inc., Milford, MA) was equilibrated in 0.1% trifluoroacetic acid (TFA, Kebo Lab) in water. The bound peptides were eluted

isocratically in a system of acetonitrile (Lab Scan) and water containing 0.1% TFA. A flow rate of 1 ml/min was used with UV detection at 220 nm.

RESULTS

Degradation of dDAVP in Gastric Juice and Intestinal Contents

The pH and protein content of the gastric juice and intestinal contents are shown in Table 2. When dissolving dDAVP in the different gastrointestinal juices, a considerable degradation was found to occur in the ileum fractions from subjects No. 2, 3, and 4 (Fig. 1). After approximately 35 min 50% of the peptide remained intact. No degradation was seen in the other fractions or in the gastric juice. The degradation was faster in a fresh specimen of intestinal contents than in a sample stored at -70°C for several months.

After adjustment of the pH to 6.5 in the duodenal and jejunal fractions from subject 2, degradation of dDAVP was found to

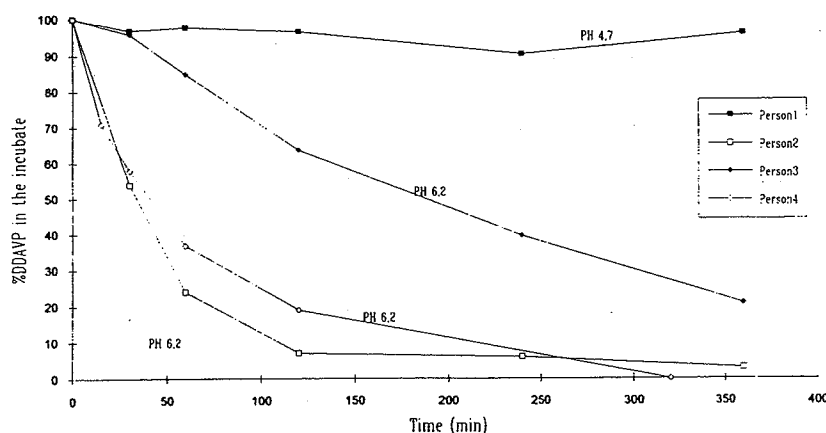


FIG. 1. Important degradation of dDAVP was seen when incubated in human intestinal contents from the ileum ($n = 3$, pH 6.2). (The sample from subject No. 1 was accidentally taken from the lower part of the jejunum, pH 4.7).

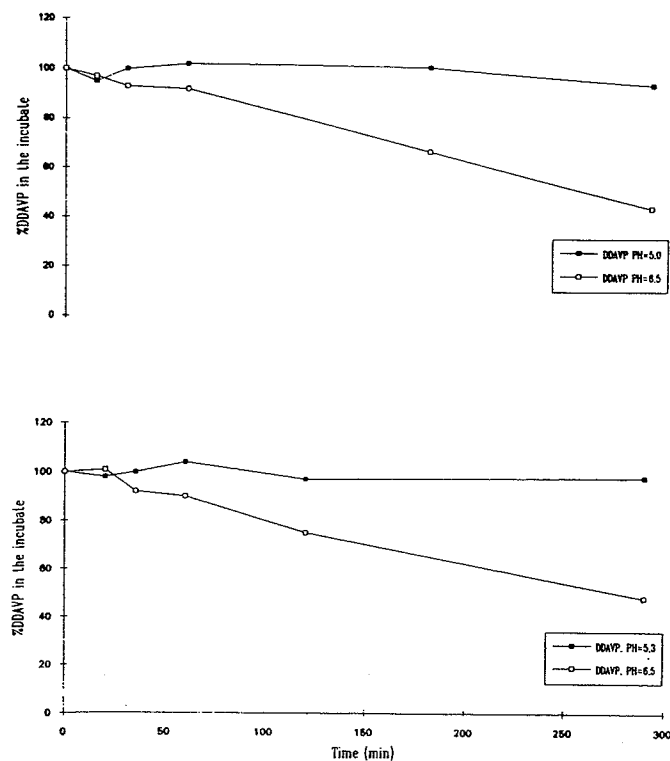


FIG. 2. Degradation of dDAVP also occurred when incubated in the duodenal (upper panel) and jejunal (lower panel) fractions in one subject (No. 2) after adjustment of the pH to 6.5.

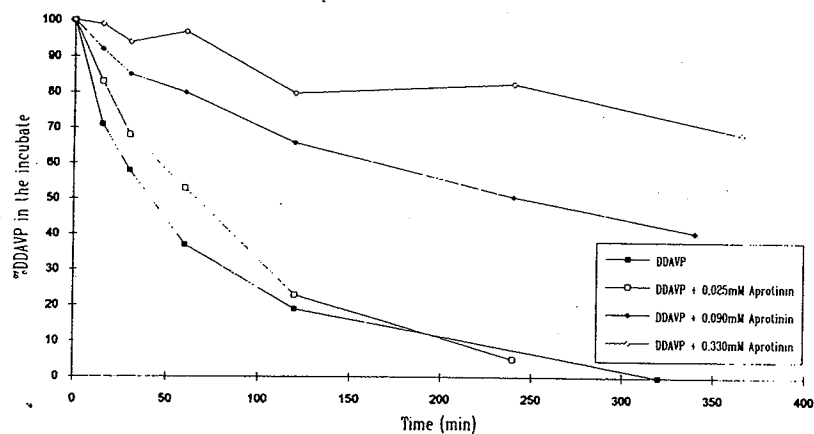


FIG. 3. Incubation of dDAVP alone in intestinal contents from the ileum and with different concentrations of an enzyme inhibitor (aprotinin) showing a concentration-dependent inhibition of the degradation of dDAVP.

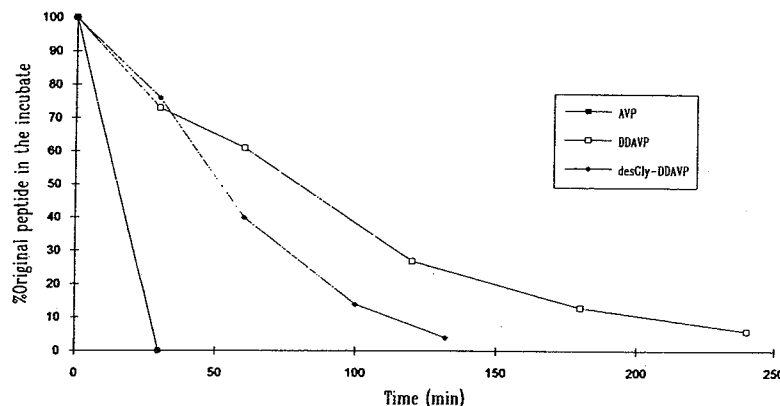


FIG. 4. Stability of some vasopressin analogues in human intestinal contents from the ileum. In comparison to dDAVP, AVP was rapidly and completely degraded within 30 min.

also occur in these fractions (Fig. 2). When lowering the pH to 4.0 in the ileum fraction, no degradation was seen. A concentration-dependent inhibition was observed when aprotinin was preincubated with contents from the ileum (Fig. 3). A similar inhibition was obtained with a more specific enzyme inhibitor (chymostatin).

Stability of Different Peptide Analogues in Human Intestinal Contents

The peptides (Table 1, lower panel) were incubated under the same reaction conditions with intestinal contents from the ileum (subject 2). The results show a marked difference in the stability between AVP and dDAVP (Fig. 4). AVP was completely degraded within 30 min.

When comparing some uterotonic peptides (Table 1), C-terminal amides seem to be more stable than acids (Fig. 5). When

analyzing the samples on HPLC, new and more hydrophilic peaks were observed in parallel with the disappearance of the original substances. The peptide ring, which consists of the first six amino acids in CAP with an amide in position six and a sulphur bridge between Mpa¹ and Cys⁶ seemed to be stable in ileal contents (from subject 3).

Metabolism of Peptides by Gastrointestinal Brush-Border and Plasma Membranes

All membrane preparations showed an enrichment of alkaline phosphatase compared to the crude homogenate: stomach ($\times 5.1$), proximal jejunum ($\times 7.4$), distal ileum ($\times 10.3$), colon ($\times 2.3$), and rectum ($\times 5.2$). Cathepsin B was undetectable, indicating absence of intracellular enzymes. Figure 6 represents a typical chromatogram of AVP incubated with homogenized ileal mucosa in the presence of glutathione during 180 min. No significant degradation was apparent until after 180 minutes.

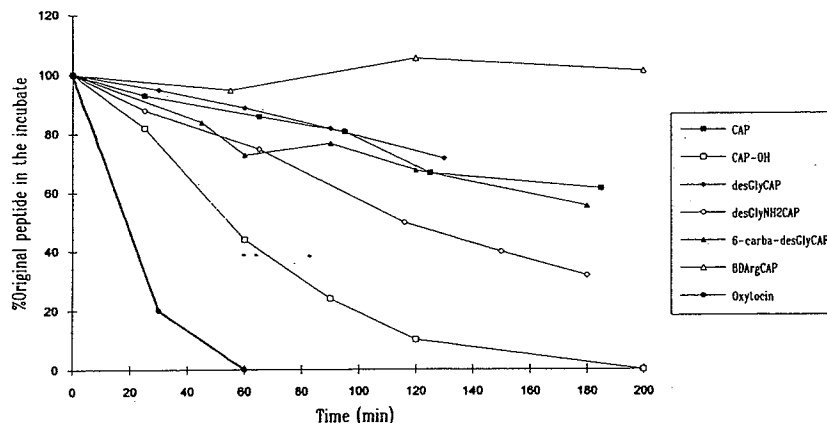


FIG. 5. Stability of different uterotonic peptide analogues in human intestinal contents from the ileum indicating that C-terminal amides seem to be more stable than corresponding acids.

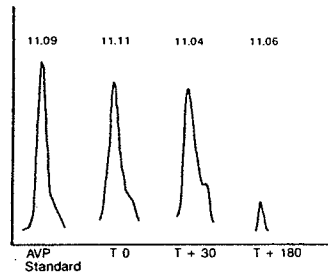


FIG. 6. HPLC chromatogram of AVP incubated in human distal ileum mucosa homogenates in the presence of reduced glutathione (10^{-4} M) for 180 min at 37°C.

In the presence of glutathione there was a degradation of oxytocin in the distal part of the ileum after 180 min. Metabolism was negligible when OT was incubated without glutathione with homogenized jejunal and ileal mucosa homogenates (Table 3).

There was a degradation of AVP and dDAVP in the presence of glutathione in the homogenized jejunal brush-border membrane occurring at 30 min. There was also a degradation occurring at 180 min in the presence of glutathione of AVP, dDAVP, and antocin in the rectal mucosa homogenates.

In the colon there was also a considerable degradation of AVP in the presence of glutathione where 40% of the initial peptide concentration is present after 30 min. Under the same conditions no degradation of AVP, dDAVP, or oxytocin analogues was observed in the mucosa homogenate from the stomach.

DISCUSSION

Earlier studies have demonstrated a rapid degradation of AVP in intestinal contents from ileum in rabbits, occurring within 5 min, although dDAVP was stable under the same conditions (17). AVP incubated in pancreatic juice from pigs (preactivated

with enterokinase) was completely degraded within 5 min whereas dDAVP remained stable for 60 min (13). The results from the present study in humans are slightly conflicting because a pH-dependent degradation of dDAVP was seen in undiluted intestinal contents collected from different parts of the small intestine, indicating an enzyme-dependent degradation. It is well known that DDAVP and related peptides are more stable at acidic pH. To assess this in more detail, the ability of aprotinin (a potent large spectrum proteinase inhibitor) to inhibit the degradation of dDAVP intestinal juice from ileum was investigated. A concentration-dependent inhibition was observed. Depending on the local pH in the small intestine the degradation of dDAVP might occur at various anatomical sites.

These results are in line with previous findings, which showed that antidiuretic (AD) activity of AVP was eliminated by trypsin and α -chymotrypsin, whereas the AD activity of dDAVP was only partly destroyed by α -chymotrypsin and unaffected by trypsin (15). Trypsin prefers to cleave L-Arg or L-Lys bonds in peptides, but dDAVP has a D-Arg instead of L-Arg in position 8, which explains the increased stability against trypsin. Oxytocin is not attacked by trypsin but may be susceptible to cleavage at several peptide bonds. These include Tyr²-Ile³, which are targets for chymotryptic activity, and Leu⁶-Gly⁷, susceptible to chymotrypsin and elastase activity (19). There are several analogues of oxytocin where modifications of the peptide sequence have been made at positions 1, 2, 4, 8, and 9. In one analogue, a carba group was substituted for sulphur at position six of the ring structure (Fig. 5). This modification did not lead to an increased stability against GI enzymes. Deletion of a C-terminal amide in favor of the free acid reduced the enzymatic stability. Ornithine at position 8 increased the resistance to tryptic cleavage whereas a D-Arg residue at this position completely abolished degradation. Furthermore, it is likely that the modifications made in the ring structure of the oxytocin molecule, namely deamination at position 1, and substitution of L-Tyr with D-Tyr (Et) at position 2, increased the resistance to chymotryptic attack.

In the present study, the natural peptides AVP and oxytocin were found to be rather slowly degraded by intestinal microvilli membranes or gastric, colonic, and rectal plasma membranes,

TABLE 3
DEGRADATION OF AVP, OT, AND ANALOGUES IN GASTROINTESTINAL MICROVILLI AND MEMBRANE PREPARATIONS ANALYZED BY HPLC

| | Stomach | | Jejunum | | Ileum | | Colon | | Rectum | |
|------------|---------|----|---------|------|-------|------|-------|------|--------|------|
| | G- | G+ | G- | G+ | G- | G+ | G- | G+ | G- | G+ |
| AVP | - | - | - | + | - | + | (+) | + | (+) | + |
| | | | | 30% | | 85% | | 40% | | 30% |
| | | | | 30' | | 180' | | 30' | | 180' |
| | | | | 70% | | | | 50% | | |
| | | | | 180' | | | | 180' | | |
| dDAVP | - | - | (+) | (+) | - | - | - | - | - | + |
| | | | | | | | | | | 30% |
| | | | | | | | | | | 180' |
| OT | | | (+) | - | (+) | + | | | | |
| | | | | | | 60% | | | | |
| | | | | | | 180' | | | | |
| Antocin II | - | - | - | (+) | (+) | - | - | - | - | + |
| | | | | | | | | | | 25% |
| | | | | | | | | | | 180' |
| Carbetocin | | | - | - | - | - | | | | |

G-, without glutathione; G+, with glutathione; - no breakdown, (+) low breakdown, + breakdown expressed in percent of initial value. Time of degradation is indicated (min).

which could be due to a number of enzymes like amino peptidase, endopeptidase 24.11 (18), and thiol:protein-disulfide oxidoreductase, which reduces disulfide bonds (4). The OT analogue carbetocin was not degraded, indicating stability towards amino peptidases. Another OT analogue (antocin) was more stable than DDAVP in intestinal microvilli membranes. Preliminary results indicate a higher absorption of antocin than DDAVP from the small intestine in pigs (unpublished results). Incubation in mucosal brush-border membranes showed degradation of AVP in the presence of glutathione in the distal part of the ileum occurring at 180 min, whereas degradation by colonic membranes was evident at 30 min. Although the activity of alkaline phosphatase was assessed in the mucosa preparations, the peptidase activity could vary depending on age and various diseases.

Both AVP and dDAVP were previously incubated with the human colorectal carcinoma cell line Caco-2 and were found to be intact after 180 min (14). This cell line differentiates into an enterocyte-like epithelium but the enzymatic expression of Caco-2 cells may be different from a normal small intestine epithelium (11).

This indicates that thiol:protein-disulfide oxidoreductase requiring glutathione may be involved in the degradation of AVP

in the intestinal mucosa in parallel with observations in kidney proximal tubule (4).

In conclusion, the degradation of dDAVP is relatively slow compared with AVP in intestinal contents, and the molecule is stable in the absence of bilio pancreatic enzymes when exposed to mucosal intestinal membrane enzyme activities. Data in this study indicate that the major enzymatic barrier to intestinal absorption of oxytocin and vasopressin and their analogues is present in the intestinal contents and not in the mucosa, which, however, constitutes a major physical barrier to peptide transport. In addition to epithelial permeability studies these degradation experiments provide valuable tools for evaluating peptides prior to costly and time-consuming *in vivo* studies.

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Errata

- page 1142, right column, line 23: "20 ml" read "100 ml"

- page 1146, left column, line 11: "40 %" read "60 %"

III

**METABOLISM OF VASOPRESSIN, OXYTOCIN AND THEIR ANALOGUES
[Mpa¹, D-Arg⁸]-VASOPRESSIN (dDAVP) AND [Mpa¹, D-Tyr(Et)², Thr⁴, Orn⁸]-
OXYTOCIN (ANTOCIN) BY HUMAN KIDNEY AND LIVER HOMOGENATES**

Metabolism of AVP and OT in human kidney and liver

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Summary

Information regarding the metabolic fate of the neurohypophyseal hormones arginine vasopressin (AVP), oxytocin (OT) and their analogues in man is practically non-existent. The aim of the present study was to investigate the stability of oxytocin, vasopressin and their analogues, dDAVP and [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-oxytocin (antocin) in human renal microvilli brush border membranes and in human liver membranes.

After incubation the extent of degradation of the peptides was determined by reversed phase high-performance liquid chromatography (HPLC). The degradation of both AVP and OT was rapid in the presence of reduced glutathione and human renal microvilli membranes. AVP, as well as dDAVP, was stable when incubated with microvilli membranes without reduced glutathione, while OT was metabolized. The metabolization of the oxytocin analogue, antocin, also varied with the presence of reduced glutathione. While in the absence of reduced glutathione a more lipophilic peak eluted, a more hydrophilic peak was observed on HPLC in the presence of glutathione. The lipophilic peak was found to coelute with the truncated analogue [Mpa¹, D-Tyr²(Et), Thr⁴, desOrn⁸, desGly⁹]-oxytocin.

No degradation occurred when the peptides were incubated with liver plasma membranes. However, when using crude, unpurified liver homogenate degradation occurred for all peptides except antocin. The degradation of AVP in the human unpurified liver homogenate was slower than in the renal microvilli membranes. Similarly OT was more rapidly degraded in human kidney microvilli membranes in the presence of reduced glutathione than in the human crude liver homogenate, when using equal amounts of protein in the incubations. Thus the present investigation indicates the existence of two possible metabolic pathways, in kidney microvilli, one for OT, which did not require the presence of reduced glutathione, and one for AVP, which required the presence of reduced glutathione. Liver degradation, on the other hand, requires uptake into the hepatocytes.

Introduction

There is scant information regarding the metabolic fate of the neurohypophyseal hormones arginine vasopressin (AVP) and oxytocin (OT) and their synthetic analogues. Most studies have been carried out in experimental animals and practically no data is available in man. The distribution of tritium-labelled AVP (1) and dDAVP (2) has been determined in rat studies where it was found that the largest amount of radioactivity had accumulated in the small intestine, liver and kidney. Beyond elimination by biliary and urinary clearance, the peptides might thus be metabolized in these organs. Previous work was mainly based on the use of kidney homogenate together with these peptides (3). Proposed mechanisms of hormone degradation have been forwarded (4, 5). The degradation of AVP by the proximal tubule of the rat was described in a detailed study by Carone et al in 1987 (6). According to these findings AVP was metabolized both by isolated brush-border membranes and by a cortical lysosomal fraction which in vitro required the presence of reduced glutathione. 1-deamino-8-D-arginine vasopressin or dDAVP, a long-acting nonapeptide, analogue of AVP, has been used administered intranasally with success in central diabetes insipidus (DI) for more than two decades (7). Using a specific radioimmunoassay for measurement of plasma dDAVP it has been shown that the intact peptide can be transported through the intestinal mucosa in man (8). A recent study indicates that the major enzymatic degradation of OT, AVP and their analogues occurs in the intestinal juice and not in the mucosa which constitutes, however, a major physical barrier to peptide transport (9). Furthermore, after oral administration dDAVP elicits an antidiuretic effect in hydrated human volunteers and in patients with DI (10-12). An antiuterotonic oxytocin analogue, [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-oxytocin (antocin) is effective in the treatment of premature labor (13-15). It is therefore important to gain insight into molecular modifications that might improve the stability of oxytocin and vasopressin in order to facilitate both penetration through mucosal barriers, intestinal and renal, and improve post absorptive stability.

In the present study we report some data on the stability of oxytocin and vasopressin and on their analogues dDAVP and [Mpa¹-D-Tyr²(Et), Thr⁴, Orn⁸]-OT (antocin) in human renal microvilli brush-border membranes, and in human liver membranes.

Key words: glutathione, membranes, microvilli brush-border, neurohypophysis

Materials and Methods

Peptides and chemicals

The molecular structures and abbreviations of the investigated peptides are shown in Table 1. All peptides were synthesized by solid phase method (Ferring Pharmaceuticals, Malmö, Sweden), and had a chromatographic purity of > 98%. Glutathione (reduced form) was purchased from Sigma.

Tissue sampling

Kidney tissue was obtained from one male patient with unilateral adenocarcinoma. After removal, the kidney was immediately bisected and chilled on ice. Cortical tissue, from a healthy section of the deceased kidney, which appeared macroscopically intact was dissected free from medulla, connective and fat tissues and was thereafter stored at -70°C until required. Liver tissue was obtained at the time of transplantation from a male person who had died in an automobile accident. The tissue was cut into pieces that were stored at -70°C until required.

Preparation of membranes

Human renal microvilli brush-border membranes were prepared according to the method described by Booth and Kenny (16). Briefly, the frozen renal specimen was thawed at room temperature and then cut into small pieces and homogenized (Polytron model PT 10/35 Kinematica) for 3 x 9 s in 100 ml ice-cold Mannitol (10 mM) Tris HCl (2 mM, pH 7.10, 4°C). Solid MgCl₂ · 6H₂O was added to give a concentration of 10 mM and the homogenate was then stirred occasionally for 15 min in an ice bath. The homogenate was thereafter centrifuged (1,500 g, 15 min, 4°C) and the supernatant was centrifuged a first time at high speed (15,000 g, 15 min). The pellets were resuspended in 100 ml buffer (Mannitol-Tris-MgCl₂) and homogenized and centrifuged (2,200 g, 15 min, 4°C). Then the supernatant was centrifuged (15,000 g, 15 min, 4°C) and the final pellet was resuspended in 5 ml buffer and briefly homogenized. Membranes were stored in aliquots at -70°C.

Human liver membranes were prepared according to a method described by Postel-Vinay et al. (17). The frozen liver specimen was thawed and homogenized (Polytron model PT 10/35 Kinematica) for 30 s in ice-cold Tris (10 mM), NaCl (0.5M), pH 7.40, 4°C. The 9,000 g (30 min) supernatant was centrifuged twice at 100,000 g for 60 min. Membranes were stored in aliquots at -70°C. Protein content was measured according to Lowry et al. (18). Crude liver homogenate was homogenized as above and was then ultracentrifuged twice (Beckman, 100,000 g for 60 min at 4°C). The pellet was resuspended in 12 ml TrisNaCl and

the homogenate was stored in aliquots of 500 µl at -70°C. The purity of the final membrane pellet was determined by measurements of membrane markers such as alkaline phosphatase (19).

Degradation in human kidney cortex microvilli and human liver membranes

AVP, dDAVP, oxytocin and antocin (Table 1) were dissolved in a medium consisting of 100 mM Tris-HCl containing 150 mM NaCl at pH 7.4. The incubation mixture consisted of 1 - 10 µg membrane protein and 10⁻⁴M of peptide in a final volume of 100 µl. Samples were prepared in duplicate. To half of the incubations, a cofactor (reduced glutathione 10⁻⁴M) was added, which has been shown to be removed in the membrane purification step (6). The incubations proceeded at 37°C in a rotating waterbath and after 0, 30 and 180 min the reaction was stopped by boiling for 5 min followed by centrifugation.

Determination of peptide degradation

Peptides were identified by HPLC employing a KONTRON HPLC-pump 420 equipped with an autosampler, and UV detector 430 and a KONTRON 460 integrator. Mobile phases were filtered through 0.45 µm filters (Schleicher & Schuell).

The column (µBondapak™ C₁₈, 300 x 3.9 mm, Waters Inc, Milford, Ma, USA) was equilibrated in 0.1% trifluoroacetic acid (TFA, Kebo Lab) in water. The bound peptides were eluted isocratically in a system of acetonitrile (Lab Scan) containing 0.1% TFA. A flow rate of 1 ml/min was used with UV detection at 220 nm. Besides synthetic standards the metabolite [Mpa¹, D-Tyr² (Et), Thr⁴, desOrn⁸, desGly⁹]-oxytocin was utilized as a standard in the same HPLC system. The recovery of peptide standards injected into the system was 100 %.

Peptide sequencing

Sequence data were obtained on an automated pulsed-liquid sequencer (Applied Biosystems Model 477A). Samples from the HPLC fractionations of AVP and oxytocin were used for sequencing, without prior reduction or alkylation. Gaps in the sequence were interpreted as cysteine since this amino acid cannot be detected by the method used.

Results

All membrane preparations showed high concentrations of alkaline phosphatase compared to the crude homogenate: kidney microvilli (x 7.0) and liver membrane (x 3.0). The liver crude homogenate containing 14.0 mg/ml protein had an alkaline phosphatase concentration of 226 U/l.

The degradation of both AVP and oxytocin was rapid when incubated with renal microvilli membranes together with glutathione (Fig. 1). Without reduced glutathione no degradation of AVP occurred whereas oxytocin was metabolized yielding a more hydrophilic peak (Fig. 2). Due to the rapid metabolism of oxytocin in the presence of reduced glutathione only two fragments could be sequenced (Table 2). Similarly, due to the rapid metabolism of AVP in the presence of reduced glutathione only two fragments could be observed as well for AVP (Table 2). dDAVP remained stable when incubated with renal microvilli membranes. The oxytocin analogue, antocin, also exhibited a different degradation with or without reduced glutathione (Fig. 3). In the absence of reduced glutathione a peak eluted on HPLC which was more lipophilic (Fig. 3), whereas in the presence of reduced glutathione a more hydrophilic peak was observed. The lipophilic peak was found to coelute with the shortened analogue [Mpa¹, D-Tyr²(Et), Thr⁴, desOrn⁸, desGly⁹]-oxytocin. This peak was also observed, although to a very limited extent, in the presence of reduced glutathione. The hydrophilic peak was not identified.

There was no degradation of any of the peptides when incubated with liver plasma membranes with or without reduced glutathione. However, when incubating the peptides in crude liver homogenate, degradation was observed for all peptides except antocin (Fig. 4). The rate of degradation was not very different for oxytocin, AVP and dDAVP.

The degradation of AVP in the human unpurified liver homogenate was slower than in the renal microvilli membranes. Similarly OT was more rapidly degraded in human kidney microvilli membranes in the presence of glutathione than in human crude liver homogenate.

Fig. 5 shows HPLC chromatogram of the degradation of AVP, dDAVP and OT incubated in human crude liver homogenate.

Discussion

Although the neurohypophyseal hormones AVP and oxytocin were among the first peptides to be sequenced and synthesized, information regarding their metabolic fate in man has been lacking. An *in vitro* inactivation study using rat kidney and liver homogenates showed that 70% of the AVP was inactivated by the liver and kidney at 30 min, while no more than 20% of the dDAVP was inactivated by the liver homogenate and little by the kidney homogenate (20). A comprehensive study of AVP metabolism in rat kidney showed that glutathione was required as a cofactor (6). It was found that AVP was degraded at both the C and N-terminus as well as by disulphide bond cleavage. Both AVP and oxytocin were incubated with pig kidney microvilli membranes without glutathione (21). Under these conditions AVP was resistant to hydrolysis whereas oxytocin was slowly degraded by cleavage of the Pro⁷-Leu⁸ bond. The principal degrading organs for AVP and oxytocin are the liver and the kidney (22). Both organs contain vasopressin-receptors, the liver the V₁ receptor subtype and the kidney the V₂ receptor subtype.

In the present study AVP and oxytocin and their analogues dDAVP and antocin were incubated with human kidney microvilli membranes. The course of degradation was different depending on whether or not reduced glutathione was present. Glutathione is removed in the process of membrane purification and must be added in *in vitro* experiments. *In vivo* glutathione may play a role for the initial splitting of disulfide bonds by reduction (23). Without reduced glutathione degradation appeared to be slower and the mechanism different. The fact that AVP was almost stable without reduced glutathione whereas oxytocin was degraded could be due to the action of the membrane-bound enzyme endopeptidase 24.11 (21) which attacks hydrophobic peptide bonds (24) and not to the post-proline enzyme described by Walter and Simmons (5) whose location is intracellular. Such a lipophilic bond is present in oxytocin at Pro⁷-Leu⁸ but not in AVP. However, a cleavage at this site was also found in antocin which is more structurally similar to AVP at the C-terminus (Pro⁷-Orn⁸). It could be that the alignment of this, more modified, molecule permits the attack of another enzyme. AVP, oxytocin and antocin were more actively degraded in the presence of reduced glutathione whereas dDAVP was found to be stable. This finding confirms the data of Carone et al (6) suggesting the activity of a glutathione-dependent oxidoreductase. The fragments identified by peptide sequencing were also originating from the peptide ring structure (Table 2). Their composition suggests that other enzymes also must have been involved. In fact, initial splitting of the Cys¹-Cys⁶ disulphide bond seems to facilitate degradation by other enzymes. We have no explanation for the high stability of dDAVP in renal membranes. In man, the plasma

clearance of this analogue is only marginally different from that of the more modified analogue antocin (25, 26). Perhaps the presence of D-Arg⁸ conveys a greater overall stability to the molecule.

When incubating the peptides with purified liver plasma membranes no degradation could be observed. The reason for this is not clear. It is well known that most of the metabolic activity in the liver is present in the lysosomal and microsomal subcellular compartments. An enzyme of aminopeptidase specificity which inactivates oxytocin has been isolated and characterized in primate liver microsomes and lysosomes (27). It is possible that oxytocin and AVP undergo receptor - mediated endocytosis with subsequent intracellular metabolism (28). This is supported by findings in the rat for AVP (29) and for dDAVP in pigs (30) where significant amounts of peptide were removed in liver perfusion experiments. In humans the plasma clearance of AVP was prolonged by steady-state infusion in cirrhotic patients (31).

To summarize, AVP and oxytocin, showed different susceptibility to degradation in human kidney microvillar membranes and liver plasma membranes. In the liver, degradation only took place in the presence of whole cellular components.

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Legends to figures

- Fig. 1. HPLC chromatogram of vasopressin (AVP) and oxytocin incubated in human kidney microvilli brush-border membranes in the presence of reduced glutathione for 180 minutes showing a rapid degradation of both peptides and appearance of hydrophilic metabolites. The retention times are indicated on the top of each peak [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].
- Fig. 2. Important degradation of oxytocin was seen when incubated in human kidney microvilli brush-border membranes without the presence of reduced glutathione at 180 min. In addition to the main metabolite of oxytocin (M) two minor hydrophilic peaks are unidentified. The retention times are indicated in the figure.
- Fig. 3. HPLC chromatogram obtained from incubation (180 min at 37°C) of Antocin with human renal microvilli membranes in the absence of reduced glutathione (10^{-4} M) showing a lipophilic metabolite at 30 and 180 minutes. The retention times of antocin and metabolite as standards are indicated in the figure [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].
- Fig. 4. Degradation of AVP (filled circle), dDAVP (hollow circle), OT (triangle) and Antocin (cube) incubated in crude liver homogenate for 180 minutes.
- Fig. 5. HPLC chromatogram of AVP, dDAVP and oxytocin incubated in human unpurified liver homogenate for 180 minutes at 37°C showing a degradation of all three peptides and appearance of a hydrophilic metabolite at 30 and 180 minutes of oxytocin. The retention times are indicated in the figure [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].

Table 1. Amino acid sequences of the peptides used for metabolism studies with disulphide structures indicated.

| Peptide | Amino acid sequence |
|----------|---|
| Oxytocin | Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-GlyNH ₂ $\begin{array}{c} \text{[S} \text{-----} \text{S]} \end{array}$ |
| Antocin | Mpa-D-Tyr(Et)-Ile-Thr-Asn-Cys-Pro-Orn-GlyNH ₂ $\begin{array}{c} \text{[S} \text{-----} \text{S]} \end{array}$ |
| AVP | Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-GlyNH ₂ $\begin{array}{c} \text{[S} \text{-----} \text{S]} \end{array}$ |
| dDAVP | Mpa-Tyr-Phe-Gln-Asn-Cys-Pro-D-Arg-GlyNH ₂ $\begin{array}{c} \text{[S} \text{-----} \text{S]} \end{array}$ |

Mpa = Mercapto propionic acid

Table 2. Amino acid sequences of fragments of oxytocin and AVP obtained after incubation in human kidney microvilli membranes in the presence of 10^{-4} M reduced glutathione. T_r denotes retention time on HPLC.

| | T_r | Peptide sequence |
|----------|-------|---|
| Oxytocin | 4 | Ile-Gln-Asn-Cys |
| | 8 | Cys-Tyr-Ile-Gln-Asn-Cys-Pro [S-----S] |
| AVP | 6,5 | Cys-Tyr-Phe-Gln |
| | 8,5 | Cys-Tyr-Phe-Gln-Asn-Cys [S-----S] |

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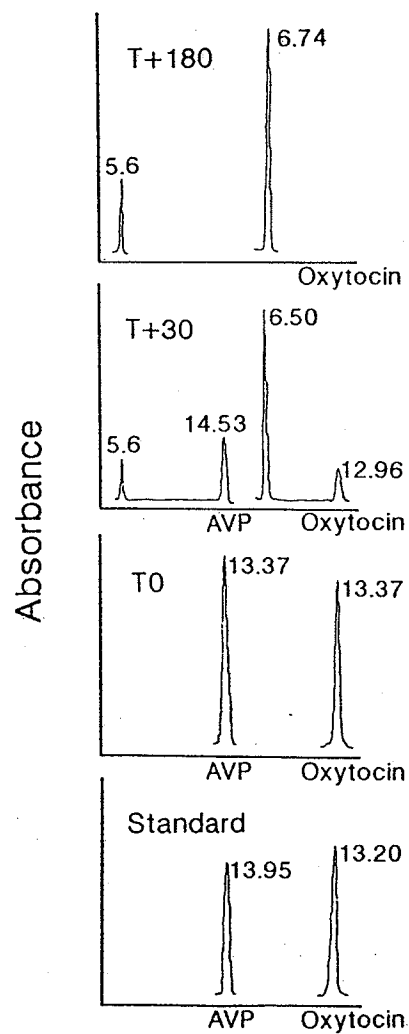


Fig. 1. HPLC chromatogram of vasopressin (AVP) and oxytocin incubated in human kidney microvilli brush-border membranes in the presence of reduced glutathione for 180 minutes showing a rapid degradation of both peptides and appearance of hydrophilic metabolites. The retention times are indicated on the top of each peak [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].

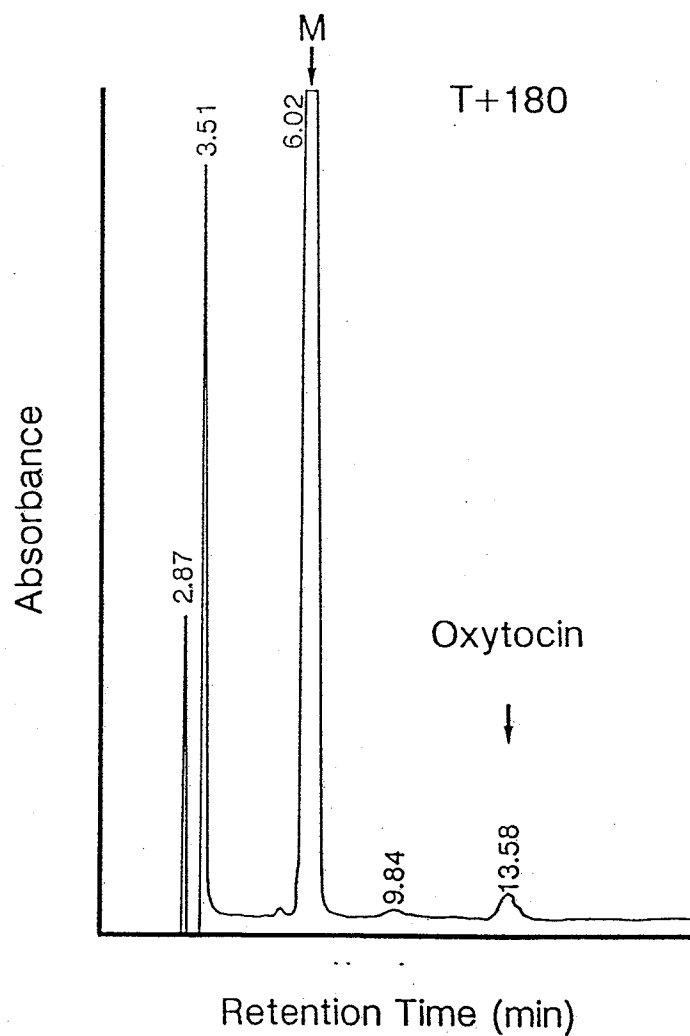


Fig. 2. Important degradation of oxytocin was seen when incubated in human kidney microvilli brush-border membranes without the presence of reduced glutathione at 180 min. In addition to the main metabolite of oxytocin (M) two minor hydrophilic peaks are unidentified. The retention times are indicated in the figure.

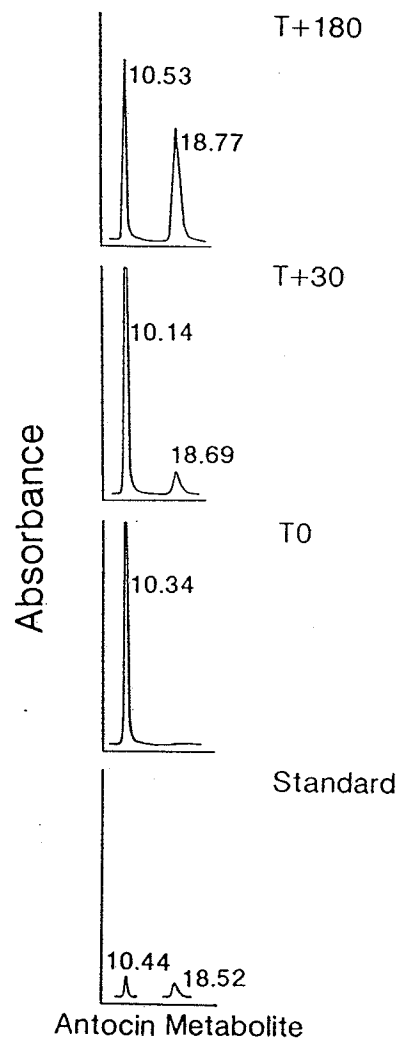


Fig. 3. HPLC chromatogram obtained from incubation (180 min at 37°C) of Antocin with human renal microvilli membranes in the absence of reduced glutathione ($10^{-4}M$) showing a lipophilic metabolite at 30 and 180 minutes. The retention times of antocin and metabolite as standards are indicated in the figure [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].

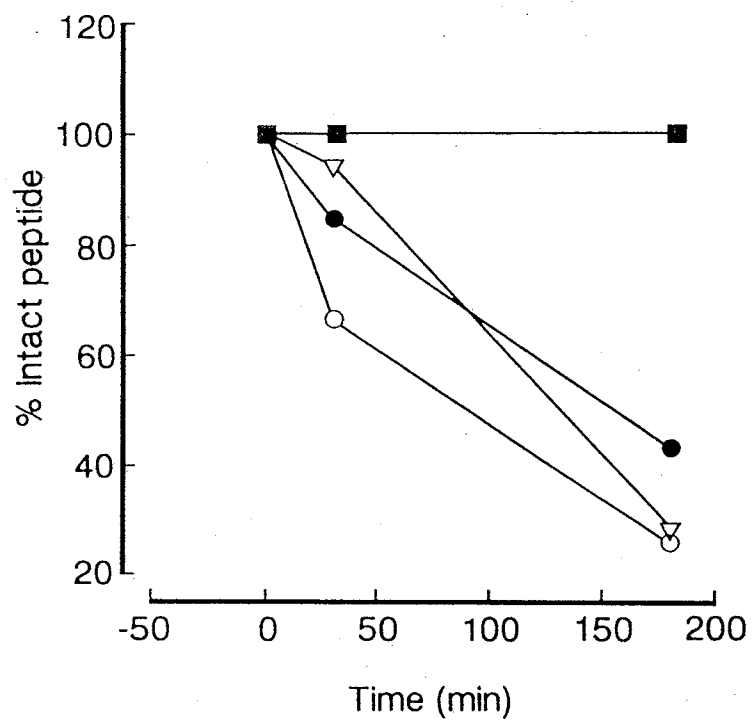


Fig.4. Degradation of AVP (filled circle), dDAVP (hollow circle), OT (triangle) and Antocin (cube) incubated in crude liver homogenate for 180 minutes.

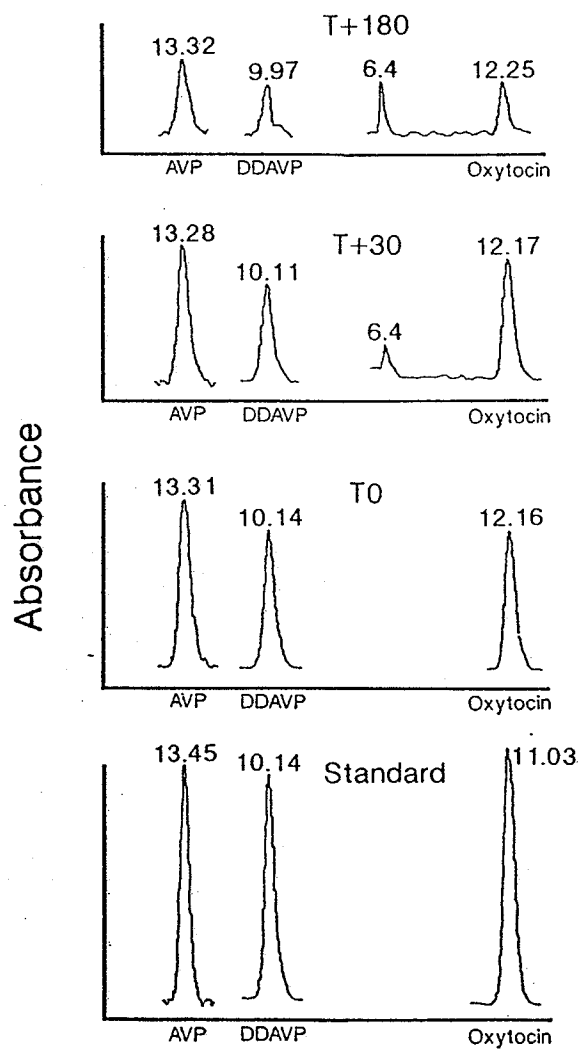


Fig. 5. HPLC chromatogram of AVP, dDAVP and oxytocin incubated in human unpurified liver homogenate for 180 minutes at 37°C showing a degradation of all three peptides and appearance of a hydrophilic metabolite at 30 and 180 minutes of oxytocin. The retention times are indicated in the figure [T0 = 0 time, T+30 = 30 min, T+180 = 180 min].

III

Pharmacokinetics of 1-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers

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Summary

OBJECTIVE We investigated the pharmacokinetics and biological effects of 1-deamino-8-D-arginine vasopressin (dDAVP) in healthy adults after intravenous, subcutaneous, intranasal, peroral, sublingual and intrarectal administration.

DESIGN Eight normal volunteers were studied over an 8-hour period after each drug administration, separated by at least one week. For intravenous and subcutaneous administration, the subjects received 2 µg of dDAVP. The intranasal and sublingual doses were 20 µg and the rectal dose was 50 µg. Oral administration of dDAVP was effected with a 200-µg tablet.

MEASUREMENTS Plasma and urinary levels of dDAVP were measured using a specific and sensitive radioimmunoassay.

RESULTS A significant increase of urine osmolality was observed after all routes of administration, except the sublingual and intrarectal for up to 8 hours after administration. After intravenous administration, the half-life of elimination ($t_{1/2}$) of dDAVP was 78 ± 10 minutes. An extensive adsorption of dDAVP to the plastic syringe was found with intravenous but not with subcutaneous administration. Using the area under the curve of dDAVP from the subcutaneous administration as a reference, bioavailability was found to be 3.4% after intranasal administration and 0.1% after oral administration. After sublingual and intrarectal routes of administration no detectable dDAVP was found in the blood; however, low amounts were found in the total 24-hour urine.

CONCLUSION The bioavailability of dDAVP seems lower

than previously reported after intranasal and oral administration.

A vasopressin analogue, the synthetic nonapeptide 1-deamino-8-D-arginine vasopressin (dDAVP) has successfully been employed in the treatment of neurogenic diabetes insipidus (DI) for more than 20 years (Andersson & Arner, 1972). dDAVP is usually administered intranasally (i.n.) (Robinson, 1976) but other modes of administration have been utilized including intravenous (i.v.), subcutaneous (s.c.) and sublingual (s.l.) administration (Edwards *et al.*, 1973; Grossman *et al.*, 1980; Laczi *et al.*, 1980). When given intragastrically or perorally (p.o.) dDAVP has been shown to give a dose-dependent antidiuretic response in hydrated dogs, in normal subjects and in patients suffering from DI (Vilhardt & Bie, 1983; 1984; Hammer & Vilhardt, 1985). Recent reports indicate that an antidiuretic effect may be obtained after rectal administration in rats (Saffran *et al.*, 1988). In a pharmacokinetic study comparing the i.n. and the p.o. routes of administration of dDAVP to children with central DI a ratio of 1:20 was found (Fjellestad-Paulsen *et al.*, 1987). The aim of the present study was to determine the pharmacokinetics of dDAVP in individual subjects where all the above-mentioned routes of administration were utilized to make both intra and inter-individual comparisons of peak levels, serum half-lives, bioavailability, and areas under the curve (AUC) of dDAVP.

Patients and methods

Subjects

Eight healthy volunteers (four female, four male), aged 20–57 years (mean 37.8), non-smokers, within 15% of their ideal weight for height (mean 65.4 kg) participated in this study. The hospital Ethics Committee had approved the study, and written informed consent was obtained from each subject prior to their entry into the study.

Laboratory methods

Blood samples (5 ml) were collected in heparinized tubes, and plasma was stored at -20°C until assay. After extraction, plasma-dDAVP was assayed with a specific dDAVP-

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radioimmunoassay (RIA) previously described (Lundin *et al.*, 1985) using an antiserum raised against 8-D-arginine vasopressin. The cross-reactivity with arginine vasopressin and oxytocin was less than 0.01%. Urinary dDAVP was measured with the same RIA method but without extraction. Urine osmolality (U-osm) was measured using an osmometer (Svenska Labex).

dDAVP

For s.c. and i.v. administration, the commercially available dDAVP solution containing 4 mg/l was employed. For i.n. and s.l. administration, a metered nasal spray, 10 µg/dose, was utilized. Oral administration was realized with commercially available tablets containing 200 µg of dDAVP. For rectal administration, methylcellulose grade 1500 AKLPM (Apoteksbolaget, Stockholm) was employed as a vehicle. Methylcellulose was dissolved in 0.9% sodium chloride (NaCl) at a concentration of 0.5% (w/v) with the addition of dDAVP to a concentration of 10 mg/l.

Drug administration

dDAVP was administered on six separate occasions to the volunteers in a randomized order and with a wash-out period of at least one week between each treatment. On arrival, the volunteers had been fasting since midnight. dDAVP was administered at 0800 h and a standardized breakfast was served 2 hours later. For s.c. administration, 2 µg of the dDAVP was given in the lateral side of the upper arm. For i.v. administration, 2 µg of dDAVP was diluted with 3.5 ml of NaCl and administered during 5 minutes. The total i.n. dose was 20 µg; 10 µg was given in each nostril with the subjects sitting upright. The total sublingual dose was 20 µg, with 10 µg administered at each side of the lingual frenulum. The rectal dose was 50 µg administered by syringe. Oral administration of dDAVP was performed with a 200-µg tablet. Blood was collected before the administration of dDAVP and at 30, 60, 90, 120, 180, 240, 300 and 360 minutes after drug administration at all occasions. In addition, blood was drawn at 5 and 15 min after i.v. drug administration and at 15 min after s.c. drug administration and also generally at 480 minutes except after i.v. and s.c. drug administration. U-osm was assessed and urine volume determined before dDAVP administration, and thereafter on 2-hour urine fractions during 6–8 hours. Determination of dDAVP in urine was made on the total amount of urine voided during 24 hours after dDAVP administration.

Pharmacokinetic calculations

dDAVP concentrations after intravenous infusion were analysed using a two-compartment model in PCNONLIN

(Statistical Consultants, Inc., 1986) (version 3.0). In all treatments the area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using the terminal elimination rate obtained from PCNONLIN. The maximum concentration (C_{max}) and the time to C_{max} (t_{max}) were the observed values.

Statistical procedure

Statistical evaluation was performed using two-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$. All data are reported as mean \pm SEM unless otherwise stated.

Results

Biological effects

In these normally hydrated healthy subjects, U-osm remained elevated during at least 6–8 hours following dDAVP administration after all routes of administration, except the i.r. route (Fig. 1). After the i.v. route, U-osm were 848 then 926 then 935 milliosmoles/kg (mOsm/kg) in the first three 2-hour urine fractions. Accordingly, diuresis remained low in the first 4–6 hours (Fig. 2). The 24-hour diuresis (Table 1) in these healthy subjects was somewhat lower (1.0–1.4 l) than normal but no difference was found between the various routes of administration.

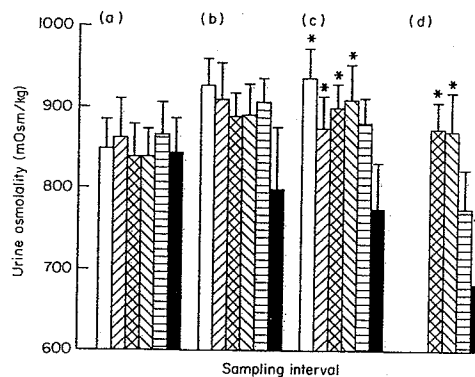


Fig. 1 U-osm (mOsm/kg) at a, 0–2; b, 2–4; c, 4–6; d, 6–8 hours after dDAVP given □, i.v. (2 µg); ■, s.c. (2 µg); ▨, i.n. (20 µg); ▩, p.o. (200 µg); ▤, s.l. (20 µg), and ▦, i.r. (50 µg). Mean \pm SEM. Differences between the various routes of administration in comparison to the i.r. route are indicated.

* $P < 0.05$.

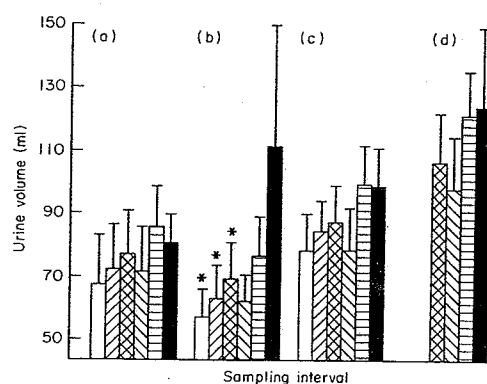


Fig. 2 U-vol (ml) at a, 0-2; b, 2-4; c, 4-6; d, 6-8 hours after dDAVP given □, i.v. (2 µg); ▨, s.c. (2 µg); ▩, i.n. (20 µg); ▤, p.o. (200 µg); ▥, s.l. (20 µg); and ■, i.r. (50 µg). Mean ± SEM. Differences between the various routes of administration in comparison to the i.r. route are indicated. * $P < 0.05$.

Table 1 24-hour urinary volume, total amount dDAVP excreted in urine and urinary clearance after various routes of administration of dDAVP in eight healthy volunteers (mean ± SEM)

| | Total amount dDAVP excreted (pmol/24 h) | Urinary clearance (ml/min/kg) | Urinary volume 24 h (ml) |
|------|---|-------------------------------|--------------------------|
| i.v. | 738 | 1.71 | 1075 ± 213 |
| s.c. | 902 | 1.30 | 1104 ± 274 |
| i.n. | 599 | 3.84 | 1156 ± 153 |
| p.o. | 122 | 1.19 | 1456 ± 323 |
| s.l. | 16 | - | 1089 ± 199 |
| i.r. | 22 | - | 1290 ± 88 |

i.v. Intravenous, s.c. subcutaneous, i.n. intranasal, p.o. peroral, s.l. sublingual, i.r. intrarectal.

Pharmacokinetics of dDAVP

The plasma concentrations of dDAVP at various intervals after the different modes of administration are shown in Fig. 3 and Table 2. Maximum levels were reached at 41.4 minutes after s.c., at 60 minutes after i.n. and at 71.4 minutes after oral administration. After i.v. administration the half-life of elimination ($t_{1/2}$) of dDAVP was 78 ± 10 minutes. Using the AUC from s.c. administration as a reference, the bioavailability was 3.4% after i.n. administration and 0.1% after p.o. administration. After s.l. and i.r. administration, no dDAVP

Table 2 Plasma dDAVP: Pharmacokinetics of dDAVP in eight healthy volunteers following i.v., s.c., i.n. and p.o. administration of dDAVP (mean ± SEM). Area under the curve, maximum concentration and time in minutes to reach this value are indicated

| Dose (µg) | AUC (pmol/l × h) | C_{max} (pmol/l) | T_{max} (min) |
|------------|------------------|--------------------|-----------------|
| i.v. (2) | 114.4 ± 10.1 | | |
| s.c. (2) | 189.4 ± 16.1 | 58.3 ± 5.0 | 41.4 |
| i.n. (20) | 58.9 ± 14.2 | 19.9 ± 3.9 | 60.0 |
| p.o. (200) | 23.8 ± 4.1 | 12.7 ± 2.1 | 71.4 |

Abbreviations as in Table 1.

could be detected in the blood, and in two of the eight subjects, plasma dDAVP was undetectable after oral administration.

Urinary excretion of dDAVP

The average amount of dDAVP (pmol/24 hours) found in urine after various routes of administration is shown in Table 1. Although no plasma dDAVP was found after s.l. or i.r. dDAVP, low but detectable amounts of dDAVP were found in 24-hour urine.

The urinary clearance of dDAVP varied from 1.19 to 3.84 ml/min/kg body weight with the different routes. Forty-eight per cent of the amount absorbed after s.c., 92% of the amount absorbed after i.n., and 65% of the amount absorbed after oral administration was excreted in the urine within 24 hours.

Discussion

The choice of the different dDAVP doses administered in this study was based upon previous pharmacokinetic studies and clinical experience in both healthy subjects and in patients suffering from DI. In these earlier investigations, 2 µg of dDAVP was given both intravenously and subcutaneously (Edwards *et al.*, 1973; Vilhardt *et al.*, 1986; Tryding *et al.*, 1987), 20 µg was administered intranasally and 200 µg perorally (Vilhardt & Lundin, 1986; Fjellestad-Paulsen *et al.*, 1987). Two authors have described an adequate antidiuretic (AD) effect with a gelatin-based sublingual lozenge containing 20 µg of dDAVP (Grossman *et al.*, 1980) and with a s.l. tablet containing 30 µg (Laczi *et al.*, 1980). In rats, 25-50% of an orally active dDAVP dose gives an adequate AD effect when given i.r. (Saffran *et al.*, 1988).

To our surprise, no dDAVP was measurable in the present study in plasma either after 20 µg given s.l. or after 50 µg i.r.

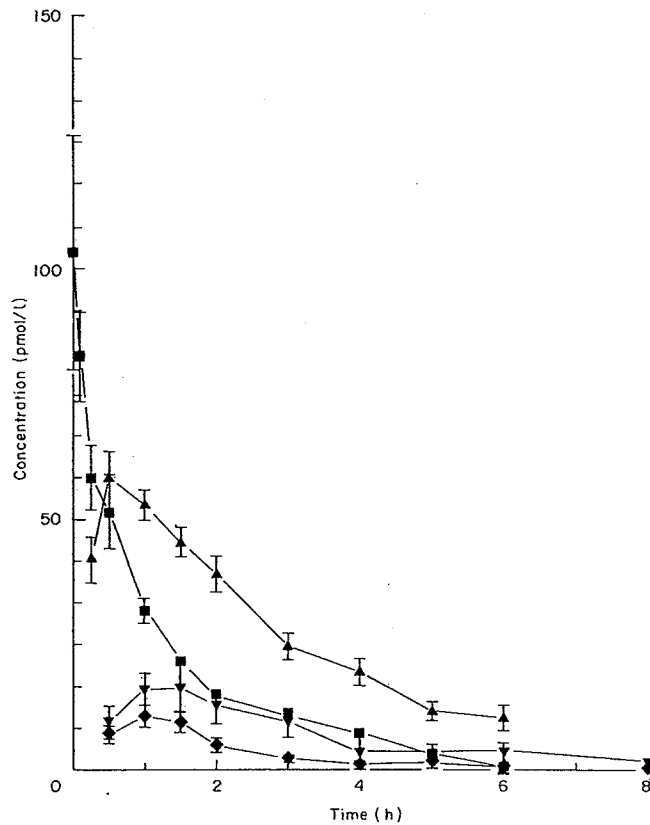


Fig. 3 Plasma dDAVP concentrations (pmol/l, mean \pm SEM) following dDAVP administered \blacksquare , i.v. (2 μ g); \blacktriangle , s.c. (2 μ g); ∇ , i.n. (20 μ g); and \blacklozenge , p.o. (200 μ g) ($n=8$).

The doses may have been too low, the sublingual mode of administration possibly inappropriate, and some amount could have been swallowed. dDAVP might be retained by the methylcellulose vehicle substance when administered intrarectally. Therefore, in an additional experiment in two of the subjects, we gave 60 μ g s.l. with a syringe and 100 μ g i.r. directly, without additive. Even so, we did not find any detectable dDAVP in blood although the U-osm was slightly increased compared to the previous experiment.

The larger AUC and the higher amount of dDAVP excreted in the urine (Tables 1 and 2) after s.c. administration than after i.v. administration is surprising and indicate that less than 2 μ g of dDAVP was infused i.v. This could be explained by a more extensive adsorption of the peptide onto the larger area of the plastic syringe utilized in i.v. administration (10 vs 1 ml). In order to assess the extent of adsorption we repeated the i.v. administration by preparing two 10-ml

plastic syringes, each containing 1 ml of dDAVP (4 mg/l) diluted in 7 ml NaCl according to the protocol. In addition, human serum albumin (HSA 0.01%) was added to the second syringe. The dDAVP content in 4 ml solution collected from the syringes was however considerably less than the expected 2 μ g, merely 1.22 μ g in the first and 1.88 μ g in the second syringe. The difference was marked and the available dDAVP was reduced by as much as 40% in the first syringe without HSA. This indicates that the peptide is adsorbed to plastic surfaces and the extent of adsorption appears to be related to the size of the area of affinity. Accordingly the bioavailability calculations in our study should be based on the AUC values seen with s.c. administration rather than those found after i.v. administration: 3.4% after i.n. administration and 0.1% after p.o. administration.

In a previous study the bioavailability of i.n. dDAVP was estimated to be 10% by comparing the antidiuretic effect

after i.n. and i.v. administration (Andersson & Arner, 1972). A more recent study showed similar results, indicating a bioavailability of 10% after i.n. administration and less than 1% after oral administration (Vilhardt & Lundin, 1986). In this study the AUC of plasma dDAVP after p.o. and i.n. administration was assessed against the plasma clearance after an i.v. dDAVP infusion. By calculating from the AUC values after i.n. and p.o. administration of desmopressin, the bioavailability after oral administration is 0.5% (Fjellestad-Paulsen *et al.*, 1987). Köhler and Harris (1988) compared the i.v., s.c. and i.n. administration of dDAVP in man. The AUC was higher after s.c. administration than after i.v. but the difference was not significant. The bioavailability after s.c. administration was 112% and after i.n. administration 2%. These authors performed two s.c. administrations with two different concentrations. The variation of the results was larger with the most diluted solution.

In the present study, the AUCs following the i.n. and the p.o. routes of administration were one-fourth of those obtained in a previous study where identical doses of dDAVP were given in the same way in children (Fjellestad-Paulsen *et al.*, 1987). After i.v. administration, we found a plasma half-life of 78 ± 10 minutes, similar to that earlier described (55 minutes) after a bolus injection of $5 \mu\text{g}$ of dDAVP (Vilhardt *et al.*, 1986). Earlier studies showed half-lives ranging between 51 (Edwards *et al.*, 1973) and 158 minutes (Pullan *et al.*, 1974) but in these studies a non-specific antiserum was used to measure the plasma dDAVP and the results must therefore be interpreted with caution. The urinary excretion of dDAVP after the various routes is more important than previously described (Vilhardt & Lundin, 1986) when expressed as a percentage of the amount absorbed (65 vs 16.4% after peroral administration).

As the main purpose of the study was to evaluate the pharmacokinetics of dDAVP after various routes of administration and as the biological effects of dDAVP are well known we did not want to investigate overhydrated subjects. Although the volunteers were fasting and had a high urine osmolality at the beginning of the study there was a measurable biological effect after administration of desmopressin. After all routes of administration, except the intrarectal, U-osm remained elevated ($> 850 \text{ mOsm/kg}$) for 6 hours although the subjects had taken breakfast at +2 hours. A more distinct biological effect would have been seen in hydrated subjects. In another study, healthy children serving as controls without medication demonstrated a spontaneous decreased U-osm (150 mOsm/kg) 2 hours after a standardized breakfast (unpublished data).

In conclusion, the bioavailability of dDAVP according to our data seems lower than previously described after intranasal and oral administration. We found a significant increase

of urine osmolality after all routes except the sublingual and intrarectal up to 8 hours after administration. No detectable dDAVP was found in blood after sublingual and intrarectal routes. We found an extensive adsorption to plastic surfaces after dilution of the solutions used for parenteral administration.

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Errata

- page 178, left column, line 25: "2 µg of the dDAVP" read "2 µg of DDAVP"
- page 179, right column, line 22: "Vilhardt et al 1986" delete

IV

Absolute bioavailability of an aqueous solution of 1-deamino-8-D-arginine vasopressin from different regions of the gastrointestinal tract in man

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Summary. The absolute bioavailability of an aqueous solution of 1-deamino-8-D-arginine vasopressin (dDAVP) from different regions of the gastrointestinal (GI) tract (stomach, duodenum, jejunum, ileum, colon, rectum) has been studied in 6 healthy, male volunteers aged 24 to 35 years, followed for 12 h after each drug administration. For i. v. administration the subjects received 4 µg dDAVP. For intestinal administration 400 µg dDAVP was directly applied to six distinct sites in the GI tract via two or four channel tubes with or without a distal occlusive balloon. Biological effects were assessed and plasma and urinary levels of dDAVP were measured using a specific, sensitive RIA.

Urine osmolality remained elevated and diuresis decreased for 12 h following dDAVP administration irrespective of the site of application. After i. v. administration, the half-life of elimination of dDAVP was 60.0 min, plasma clearance 1.7 ml · min⁻¹ · kg⁻¹, amount excreted in urine 2.0 µg and renal clearance was 0.8 ml · min⁻¹ · kg⁻¹.

The mean bioavailability (f) after gastric application was 0.19% (range 0.02–0.35%). f was 0.24% after duodenal application (range 0.04–0.62%), 0.19% after jejunal (range 0.01–0.41%), 0.03% after distal ileal (range 0.01–0.08%), 0.04% after proximal colonic (range 0.01–0.12%) and 0.04% after rectal (0.01–0.10%) application. The bioavailability was significantly higher in the three upper GI regions in comparison to the three lower regions.

The bioavailability of dDAVP after gastric, duodenal and jejunal application was similar to that after swallowing a tablet in a previous study. Absorption from the ileum was lower than expected and no preferential site of absorption was found.

Key words: dDAVP; bioavailability, gastrointestinal tract, healthy volunteers, pharmacokinetics

1-Deamino-8-D-arginine vasopressin or dDAVP, a nonapeptide and long-acting analogue of arginine vasopressin (AVP), has been used intranasally (IN) with success in central diabetes insipidus (DI) for more than two decades [Andersson and Arner 1972, Seif et al. 1978]. dDAVP is

absorbed from the gastrointestinal (GI) tract after oral administration and elicits an antidiuretic effect in conscious, water-loaded dogs [Vilhardt and Bie 1983], in hydrated human volunteers [Vilhardt and Bie 1984] and in patients with DI [Hammer and Vilhardt 1985]. Using a specific radioimmunoassay to measure plasma dDAVP, it has been shown that the intact peptide is transported through the intestinal mucosa in the rat [Lundin et al. 1985]. The efficacy and safety of oral dDAVP in children with DI was demonstrated in a long-term study [Fjellestad and Czernichow 1986]. The ratio of 1:20 between the IN and oral route of dDAVP was found in a pharmacokinetic study comparing the two modes of administration in children with neurogenic DI [Fjellestad-Paulsen et al. 1987]. Thus, the bioavailability of the oral route is low, 0.5% or less. It has been shown in rabbits that a preferential area of absorption of dDAVP may be located in the ileocaecal region [Lundin and Vilhardt 1986]. If the duodeno-ileal gradient were also to occur in man, the bioavailability of dDAVP by the oral route could be increased by using slow-release galenical forms that delivered a large part of their contents in the ileal lumen. In the present study we have assessed the bioavailability of dDAVP by applying an aqueous solution of dDAVP directly to various sites in the GI tract in healthy male volunteers, and any possible site of preferential absorption of the drug was sought.

Materials and methods

Subjects

After giving written informed consent, six healthy male volunteers, aged 24 to 35 y (mean 27.2 y), within 15% of their ideal bodyweight for height (mean = 71.0 kg), participated in this open study, which was approved by the Hospital Ethics Committee.

Laboratory methods

Blood samples (3 ml) were collected in tubes containing K₃-EDTA and plasma was stored at -70°C until assay. After acetone/petroleum ether extraction, plasma dDAVP levels were measured by

Table 1. 24 h urine volume after direct application of dDAVP to various sites in the GI tract in normal volunteers ($n = 6$; mean with (SD))

| Route of delivery | Diuresis (ml/24 h) |
|-------------------|--------------------|
| Intravenous | 696 (221) |
| Stomach | 1004 (437) |
| Duodenum | 901 (482) |
| Jejunum | 901 (160) |
| Ileum | 869 (480) |
| Colon | 1279 (823) |
| Rectum | 954 (434) |

radioimmunoassay (RIA), using a previously characterized antiserum specific for dDAVP [Lundin et al 1985]. The minimum quantifiable concentration (MQC) of the assay was set at 2.5 pg dDAVP \cdot ml $^{-1}$ plasma. The intra- and inter-assay coefficients of variation were 18.1 (9.6) % and 33.0 % at 3 pg dDAVP \cdot ml $^{-1}$, 10.8 (4.8) % and 20.4 % at 10 pg dDAVP \cdot ml $^{-1}$, 4.7 (1.7) % and 4.2 % at 100 pg dDAVP \cdot ml $^{-1}$, respectively.

Urine samples were extracted on Bond Elut C8 silica columns (LRC series, Analytichem), as described by van de Heyning et al. (1991). After extraction, urine dDAVP levels were measured by the same RIA method. The MQC was set at 5.0 pg dDAVP \cdot ml $^{-1}$ urine. The intra and interassay coefficients of variation were 12.9 (8.7) % and 16.4 % at 10 pg dDAVP/ml, 5.5 (3.1) % and 8.7 % at 100 pg dDAVP/ml, 4.7 (1.7) % and 11.2 % at 1000 pg dDAVP \cdot ml $^{-1}$, respectively. Urine osmolality (U-osm) was measured using an osmometer (advanced Microsmometer Mod 3MO).

Desmopressin

For i.v. administration the commercially available dDAVP solution containing 4 μ g \cdot ml $^{-1}$ was employed (MINIRIN[®]), and for gastrointestinal application lyophilized dDAVP with a chromatographic purity greater than 99 % (Ferring Pharmaceuticals, Malmö, Sweden) was used.

Intubation

The intraluminal perfusion employed a modification of a previously described method [Modigliani et al. 1973]. The drug was directly applied to six distinct sites in the GI tract: stomach, duodenum, mid-jejunum, distal ileum, ascending colon and rectum. The rectal application was done with a rectal canula. For all other sites, subjects were intubated via a nostril with multichannel polyvinylchloride tubes (diameter 2 mm) made by the investigator. Except for the colon, an occluding balloon was present 2 cm distal to the application point. Before drug application, at each session, the correct location of the tube was verified fluoroscopically and occlusion and the absence of leakage were confirmed by instillation of 1 ml bromosulphophthalein (BSP) as a marker.

Drug administration

Each volunteer participated in seven study sessions, separated by at least 48 h. For calculation of the AUC after i.v. administration, a bolus injection of 4 μ g dDAVP was given at 09.00 h after an overnight fast. In all other sessions 400 μ g dDAVP acetate was dissolved in 2 ml NaCl 0.9 % and was given by the appropriate route at 09.00 h, followed by rinsing of the tube with 2 ml NaCl. The occlusive balloon was deflated 1.5 h after drug application and a standardised meal was given 1.5 h later.

Blood samples for determination of the plasma concentration of dDAVP were taken before and up to 8 h after drug application. Urine

samples were collected over a 24 h period. From 2 h to 8 h following the dose, the volunteers received 100 ml water per h. Thereafter they were allowed an additional fluid intake of 500 ml until 09.00 h on the next morning. Xanthine-containing beverages and alcohol were avoided throughout the study day and smoking was forbidden.

Pharmacokinetic calculations

dDAVP concentrations after the intravenous infusion were analysed using a two-compartment model in PCNONLIN [Statistical Consultants 1986; version 3.0]. In all treatments the area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using the terminal half-life obtained from PCNONLIN. Total clearance after i.v. administration was calculated as dose divided by AUC, and renal clearance after i.v. administration as the amount excreted in urine divided by the AUC. The clearances were normalised using the body weight of each subject. Absorption bioavailability (f) was calculated as the normalised ratios between the AUCs for each segment of the GI-tract and the AUC after i.v. administration.

Statistical procedure

Statistical evaluation used analysis of variance (ANOVA). The level of significance was set at $P < 0.05$. All data are reported as mean with (SD) unless otherwise stated.

Results

Biological effects

U-osm remained elevated for at least 8–12 h following dDAVP administration in these normally hydrated volunteers irrespective of the site of application, although normal water intake was allowed 3 h after drug administration. Concomitantly, diuresis remained decreased for 12 h. The 24 h diuresis was lower (0.696–1.28 l) than normal (Table 1) [Carver and Paska 1961].

Pharmacokinetics of dDAVP

The plasma concentrations of dDAVP (p-dDAVP) at various intervals after various modes of administration in the same subject are shown in Fig. 1. After i.v. administration, the half-life of elimination ($t_{1/2}$) of dDAVP was 60.0 (14.7) min and plasma clearance was 1.7 (0.7) ml \cdot min $^{-1}$ \cdot kg $^{-1}$. The amount excreted in urine after i.v. administration was 2.0 (0.4) μ g. Renal clearance was 53.6 (19.9) ml \cdot min $^{-1}$ or 0.8 (0.3) ml \cdot min $^{-1}$ \cdot kg $^{-1}$.

Individual AUCs of dDAVP after application at various sites in the GI tract are shown in Table 2. The peak plasma concentration (C_{max}) of dDAVP in all study sessions occurred before the balloon was deflated, except for the intragastric route in one subject. In him very little absorption was observed after intragastric application, and C_{max} occurred after the balloon was deflated at 180 min. Each site of absorption was studied separately, as intended, and the absorption phase could therefore be considered to have terminated before deflation of the balloon. In one of the subjects (No.6) blood samples 5, 10, and 15 min after i.v. administration were missing. The AUC in

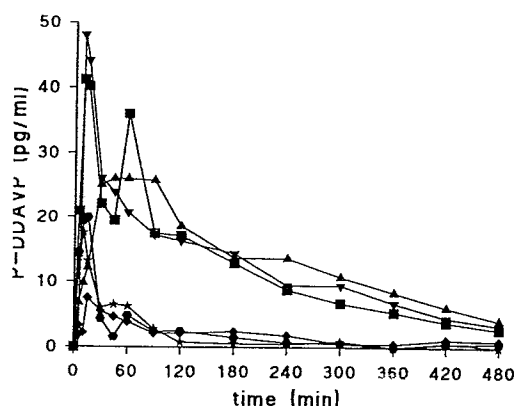


Fig. 1. Plasma dDAVP concentrations (pg/ml, mean, $n = 6$) following dDAVP 400 μ g administered in the stomach ■, duodenum ▲, jejunum ▼, ileum ◆, colon ★ and rectum ●.

that case was calculated by extrapolation and is probably underestimated. The mean bioavailability (f) after the gastric application was 0.19% (range 0.02–0.35%), 0.24% after duodenal administration (range 0.04–0.62%), 0.19% after jejunal (range 0.01–0.41%), 0.03% after distal ileal (range 0.01–0.08%), 0.04% after proximal colonic (range 0.01–0.12%) and 0.04% after rectal (range 0.01–0.10%) application (Table 2). The bioavailability was significantly higher from the three upper than the three lower regions ($P < 0.05$) of the GI tract.

Discussion

The potential therapeutic usefulness of peptides and protein drugs would be increased if other routes of delivery than the parenteral could be used. The limiting factor is the very low absorption rate and bioavailability of most pep-

tides from most non parenteral routes. However, dDAVP has been safely and efficiently used by intranasal administration for more than twenty years in DI patients [Andersson and Arner 1972; Seif et al. 1978], and orally for more than 9 years [Hammer and Vilhardt 1985; Fjellestad and Czernichow 1986]. Using a highly specific and sensitive antibody for dDAVP, the bioavailability after IN administration was found to range from 2% to 10% [Köhler and Harris 1988; Vilhardt and Lundin 1986]. In a recent study, in which six routes of delivery (IV, SC, IN, PO, sublingual and intrarectal) were compared in healthy volunteers, the bioavailability of the IN route was 3.4% [Fjellestad-Paulsen et al. 1993]. In the same study the bioavailability of the oral route was 0.1% after a 200 μ g tablet. Earlier studies had indicated higher bioavailability after oral administration of dDAVP (0.5–1.0%), but they were based on comparison of the AUC between the oral and IN routes [Vilhardt and Lundin 1986; Fjellestad-Paulsen et al. 1987].

Two studies in animals have indicated a possible area of preferential absorption of dDAVP in the distal part of the ileum in some species. Lundin and Vilhardt showed in 1986 that plasma concentrations of dDAVP in rabbits were 10-times higher after administration in the stomach, colon or in the mid-part of the ileum. An increased rate of transport of dDAVP and other peptides was found in the distal part of the small intestine in rats [Lundin et al. 1991] in a experiment using isolated segments from the proximal and distal small intestine.

In the present study the half-life of elimination of dDAVP after i.v. administration was 60.0 (14.7) min, which is in accordance with previous studies in which a specific antiserum was used to measure plasma dDAVP [Vilhardt et al. 1986; Fjellestad-Paulsen et al. 1993].

The bioavailability after administration of 400 μ g dDAVP into the stomach and upper part of the small intestine was 0.19–0.24%, which is in accordance with the results after a 200 μ g tablet given orally to healthy volunteers [Fjellestad-Paulsen et al. 1993]. Contrary to animal studies, the bioavailability after application in the distal part of the ileum was much lower (0.03%) than in the upper part of the GI tract.

Table 2. AUC and bioavailability (f) of dDAVP directly applied to various sites in the GI tract in six healthy volunteers

| Route of delivery Dose dDAVP/subject | 1 | 2 | 3 | 4 | 5 | 6 | Méan | SD |
|---|--------|--------|--------|--------|--------|--------|--------|--------|
| AUC (pg · min/ml) | | | | | | | | |
| Intravenous (4 μ g) | 36.700 | 66.800 | 28.000 | 64.700 | 25.400 | 26.050 | 41.300 | 19.400 |
| Stomach (400 μ g) | 13.000 | 1.240 | 7.190 | 3.250 | 3.000 | 8.640 | 6.060 | 4.380 |
| Duodenum (400 μ g) | 5.540 | 4.450 | 6.048 | 2.710 | 8.770 | 16.300 | 7.300 | 4.830 |
| Jejunum (400 μ g) | 3.420 | 27 | 6.450 | 10.400 | 6.370 | 10.600 | 6.210 | 3.720 |
| Ileum (400 μ g) | 1.400 | 1.390 | 840 | 190 | 535 | 2.130 | 1.080 | 701 |
| Colon (400 μ g) | 1.620 | 334 | 640 | 83 | 3.100 | 546 | 1.050 | 1.130 |
| Rectum (400 μ g) | 1.760 | 325 | 607 | 189 | 1.660 | 2.490 | 1.170 | 931 |
| (%) | | | | | | | | |
| Stomach | | | | | | | | |
| Duodenum | 0.35 | 0.02 | 0.26 | 0.05 | 0.12 | 0.33 | 0.19 | 0.14 |
| Jejunum | 0.15 | 0.07 | 0.22 | 0.04 | 0.35 | 0.62 | 0.24 | 0.22 |
| Ileum | 0.09 | < 0.01 | 0.23 | 0.16 | 0.25 | 0.41 | 0.19 | 0.14 |
| Colon | 0.04 | 0.02 | 0.03 | < 0.01 | 0.02 | 0.08 | 0.03 | 0.03 |
| Rectum | 0.04 | 0.01 | 0.02 | < 0.01 | 0.12 | 0.02 | 0.04 | 0.04 |
| | 0.05 | 0.01 | 0.02 | < 0.01 | 0.07 | 0.10 | 0.04 | 0.04 |

Absorption from the proximal part of the colon and the rectum was very low, but it was still higher than expected, and was similar to that in the distal part of the ileum. Our knowledge of large intestinal peptide absorption is limited, especially in humans.

Saffran et al. (1988) reported a good antidiuretic effect in rats when 25–50% of an orally active dose of dDAVP was given intrarectally (IR). In contrast, no measurable p-dDAVP was seen after IR administration in a recent study in healthy volunteers [Fjellestad-Paulsen et al. 1993], but the doses may have been too low (50 and 100 µg). In the present study, measurable absorption of dDAVP was found after 400 µg administered intrarectally.

In conclusion, the bioavailability of dDAVP after gastric, duodenal and jejunal application was similar to that after a swallowed tablet. Absorption after application in the distal ileum was lower than in animals and we did not find any distal site of preferential absorption. The low bioavailability of dDAVP could be consequence of intraluminal proteolysis. It has been observed in vitro that dDAVP proteolysis was slower in jejunal than in ileal juice and that the difference was due to the lower jejunal pH (unpublished). The bioavailability might then be increased if the dDAVP molecules were to be maintained intact during the absorption process. It would be of interest to study the proteolytic effect of intestinal enzymes on dDAVP, especially in a study using an enzyme inhibitor added to the dDAVP.

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Errata

- page 474, left column, line 28: "rectal canula" read "rectal cannula"
- page 475, right column, line 23: "after administration in the stomach" read "after administration in the distal part of the ileum than in the stomach"



**Bioavailability of 1-deamino-8-D-arginine vasopressin with
an enzyme inhibitor (aprotinin) from the small intestine in healthy volunteers**

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Summary

The bioavailability of an aqueous solution of 1-deamino-8-D-arginine vasopressin (dDAVP), with and without an enzyme inhibitor, has been studied in 6 healthy, male volunteers aged 19 to 34 years, followed for 8 h after each drug administration. For i.v. administration the subjects received 4 µg dDAVP. For intestinal administration 500 µg dDAVP was directly applied, in two separate sessions, in the first part of the duodenum via a triple lumen channel tube. In one session a solution of isotonic polyethylene glycol (PEG) was given as a continuing enteral administration. In the other session a solution of PEG and aprotinin was administered enterally at the constant rate of 5 ml/min and during a further four hours. Plasma dDAVP was measured using a specific, sensitive RIA and collection of intestinal juice was performed for measurement of lipase, chymotrypsin and pH every 30 min for 5 h.

The intestinal chymotrypsin activity was decreased after infusion of aprotinin ($p < 0.001$) while the lipase activity was not modified. After i.v. administration, the half-life of elimination of dDAVP was $1.56 \text{ h} \pm 0.2$ and plasma clearance $1.24 \pm 0.6 \text{ ml/min} \times \text{kg}$.

The mean bioavailability after duodenal administration of dDAVP + aprotinin was 0.46% (range 0.28 - 0.84) to compare with 0.09% (range 0.05 - 0.14) after duodenal administration of dDAVP alone ($p < 0.0148$). The bioavailability of dDAVP after direct duodenal application of an aqueous solution was similar to that after swallowing a tablet in a previous study and increased five times when given together with an infusion of an enzyme inhibitor.

Key words: aprotinin, bioavailability, dDAVP, enzyme inhibitor, gastrointestinal tract, healthy volunteer, pharmacokinetics

Current therapy of neurogenic diabetes insipidus successfully employs a synthetic nonapeptide, a long-acting analogue of arginine-vasopressin (AVP). 1-deamino-8-D-arginine vasopressin or dDAVP has been used clinically for more than 20 years (Andersson & Arner 1972) and is usually administered intranasally. Peptides are generally poorly absorbed but dDAVP given orally elicits a dose dependent antidiuretic effect in patients with diabetes insipidus (Hammer & Vilhardt 1985). A ratio of 1:20 in bioavailability was found in a pharmacokinetic study comparing intranasal and oral administration of dDAVP in children with central diabetes insipidus (Fjellestad-Paulsen et al. 1987). The efficacy and safety of dDAVP administered orally in children with diabetes insipidus has been shown in a long term study (Fjellestad-Paulsen et al. 1993a). The bioavailability of dDAVP was 0.1% after oral administration in a pharmacokinetic study in healthy volunteers (Fjellestad-Paulsen et al. 1993b). Direct application of an aqueous solution of dDAVP in the duodenum and the jejunum showed an absolute bioavailability of 0.2% (d'Agay-Abensour et al. 1993). This low bioavailability of dDAVP could be due to intraluminal proteolysis or mucosal degradation.

Recently it has been shown in an in vitro study that dDAVP proteolysis was slower in jejunal than in ileal human secretion due to a lower jejunal pH. This degradation was inhibited after concomitant administration of the enzyme inhibitor aprotinin (Fjellestad-Paulsen et al. 1995).

In the present investigation absorption of dDAVP from the small intestine with and without an intestinal perfusion of aprotinin was studied in healthy volunteers in order to evaluate the proteolytic effect of intestinal enzymes on dDAVP.

Materials and Methods

Subjects

Six healthy male volunteers, aged 19 to 34 years (mean 26.5 y) participated in the trial. They were within 15% of their ideal bodyweight for height (mean = 65.8 kg). Xanthine beverages, alcohol and smoking were avoided 12 h before each study session and throughout the study day. Approval of the study was obtained from the Hospital Ethics Committee and written informed consent was obtained from each subject prior to entering the trial.

Intubation

The subjects were intubated via a nostril with a triple-lumen channel tube of polyvinyl chloride in two study sessions (Fig. 1). The tip of the first tube, for the tractor balloon, was positioned after the duodeno-jejunal junction (ligament of Treitz) and that of the second, used for the intestinal drug application and perfusion of the solutions, was placed in the first part of the duodenum (D₁). The tip of the third tube, positioned 30 cm beyond the second, was directed to collect the intestinal juice. Before drug application, at each session, the correct location of the tube was verified fluoroscopically.

Peptides

For i.v. administration the commercially available dDAVP solution containing 4 µg/ml was employed (Minirin) and for duodenal application a lyophilized dDAVP was used. dDAVP was synthesized by solid phase method (Ferring Pharmaceuticals, Malmö, Sweden) and had a chromatographic purity of 99%. The wide spectrum proteinase inhibitor (aprotinin = Antagosan®) was purchased from Hoechst laboratories. One ampoule of 10 ml with 50 European Pharmacopeic Units contains 100 000 kallikrein inhibitor units, 800 000 protease inhibitor units, 2 500 anti-plasmin units and 167 trypsin inhibitor units.

Drug administration

The enteral administration technique was described by Modigliani and al. (1973). The subjects fasted for 12 h before each study session. Each volunteer participated in three study sessions separated by at least 48 hours. For calculation of area under the curve (AUC) after intravenous administration a bolus injection of 4 µg of dDAVP was given at 9.00 a.m. after an overnight fast. In the two other sessions 500 µg of dDAVP acetate were dissolved in 2 ml of NaCl 0.9% and administered by tube at 9.00 a.m., with subsequent rinsing of the tube with 2 ml of NaCl. A standardized breakfast was given 5 - 10 min after

drug application and a standardized meal 5 hours later. In one session, a solution of PEG (isotonic polyethylene glycol), a non absorbable marker (pH = 6.5, osmolality = 305 mOsm/kg) was given as a continuing enteral administration by a motor driven syringe starting 30 min before the dDAVP application and during further 5.5 h at the constant rate of 5 ml/min. In the other session, a solution of PEG and aprotinin was administered enterally at the constant rate of 5 ml/min and during a further 5 hours without changing pH or osmolality of the solution.

Blood samples for determination of plasma concentrations of dDAVP were drawn before the administration of dDAVP and at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes after drug administration at all occasions. Collection of intestinal juice was performed for measurements of lipase, chymotrypsin and pH at baseline and thereafter every 30 min for 5 hours. After centrifugation the intestinal juice was frozen in aliquots of 1 ml and stored at -70°C. From 2 hours until 8 hours following drug application, the volunteers received a maximum of 100 ml of water per hour. Thereafter they were allowed an additional fluid intake of 500 ml until 9.00 a.m. the next morning.

Laboratory methods

Blood samples (3 ml) were collected in tubes containing K₃-EDTA and plasma was stored at -70°C until assay. Before analysis all samples were extracted with acetone and petroleum ether. Dry extracts were stored at -20°C. Plasma dDAVP levels were measured with a radioimmunoassay (RIA) technique, using a previously characterized antiserum specific for dDAVP (Lundin et al. 1985). The minimum quantifiable concentration (MQC) of the assay was set at 2.5 pg dDAVP/ml plasma. The intra- and interassay coefficients of variation of spiked EDTA plasma were 5.9±7.2 % and 17.2 % at 5 pg dDAVP/ml, 6.1±4.2 % and 9.7 % at 10 pg dDAVP/ml, 2.3 %± 1.5 % and 3.2 % at 100 pg dDAVP/ml, respectively. The recovery of dDAVP from spiked EDTA plasma was 89.2 % at 5 pg dDAVP/ml, 77.1 % at 10 pg dDAVP/ml and 63.8 % at 100 pg dDAVP/ml. Determination of pH in intestinal juice was performed with a pH-meter (Radiometer Tacussel). PEG concentrations were measured in the intestinal juice turbidimetrically by Hyden's technique (Hyden 1955) in order to obtain the output of water at the sampling point according to the equation:

$$\text{H}_2\text{O output} = \frac{\text{Perfusion output} \times \text{PEG}_{\text{Base}}}{\text{PEG}_{\text{collected}}}$$

Chymotrypsin activity of the intestinal juice was determined titrimetrically with acetyl-tyrosine-ethylester as substrate (Figarella et al. 1965). Lipase activity was estimated similarly with emulsified olive oil as substrate (Marcuis-Mouren et al. 1959).

Pharmacokinetic calculations

Enzymes activity were determined through multiplying their concentration by water output.

dDAVP kinetics after intravenous infusion were analysed using a two-compartment model in PCNONLIN (Statistical Consultants, Inc. 1986) (version 3.0). In all treatments the area under the plasma concentration versus time curve (AUC) was calculated using the trapezoidal rule, with extrapolation to infinity using the terminal elimination rate obtained from PCNONLIN. Clearance, half life, of elimination ($t_{1/2}$) the absolute and relative bioavailability of dDAVP and the mean residence times after intravenous and intestinal administrations of dDAVP were calculated using standard formulas (Gibaldi Perrier 1982). The absorption rate was calculated by the Loo-Riegelman method (Loo and Riegelman, 1968).

Statistical procedure

Statistical evaluation was performed using paired t-tests. The level of significance was set at $p < 0.05$. All data are reported as mean \pm SD unless otherwise stated.

Results

Lipase and chymotrypsin response

The intestinal lipase activity over 4 hours was not modified by aprotinin (Table 1). In contrast, the chymotrypsin activity was significantly decreased after infusion of aprotinin ($p < 0.001$).

Fig. 2 represents the mean intraluminal chymotrypsin activity with and without aprotinin. Median hydrogen ion concentration of the intestinal juice at the jejunal aspiration site was not modified by aprotinin (pH 5.73 without and 5.75 with aprotinin).

Pharmacokinetics of dDAVP

Mean plasma concentrations of dDAVP at various intervals after an i.v. bolus injection of dDAVP (4 μ g), and after 500 μ g dDAVP acetate given either alone or with aprotinin in the duodenum are shown in Fig 3. After i.v. administration the half-life of elimination ($t_{1/2}$) of dDAVP was $1.56 \text{ hrs} \pm 0.2$ and plasma clearance $1.24 \pm 0.16 \text{ ml/min} \times \text{kg}$. The plasma samples from one patient (EMPL) after duodenal administration of dDAVP alone were excluded from the analysis because the samples were contaminated before the assay. Individual results from area under the curve (AUC) of dDAVP after duodenal administration are shown in Table 2. The mean bioavailability (F) after duodenal administration of dDAVP + aprotinin was 0.46% (range 0.28 - 0.84) to compare with 0.09% (range 0.05 - 0.14) after duodenal administration of dDAVP alone ($p < 0.0148$). AUC and F were significantly higher after duodenal administration of dDAVP with aprotinin in comparison to after duodenal administration of dDAVP alone.

The mean residence times after i.v. administration, with and without aprotinin were 1.4, 3.4 and 2.9 hours, respectively. The difference between the mean residence times with and without aprotinin was not significant.

The absorption rate constant obtained when dDAVP was given alone was $0.56 \pm 0.11 \text{ 1/h}$ and when given together with aprotinin $0.44 \pm 0.23 \text{ 1/h}$. The difference was not significant.

Discussion

Oral delivery of peptides is certainly the most convenient mode of administration in therapy. Although the gastrointestinal absorption rate and bioavailability are very low dDAVP (1-deamino-8-D-arginine vasopressin) has been administered safely and efficiently orally in the treatment of neurogenic diabetes insipidus patients for more than 10 years (Hammer & Vilhardt 1985, Fjellestad & Czernichow 1986).

In a pharmacokinetic study in which six routes of delivery (i.v., s.c., intra-nasal, oral, sublingual and intrarectal) were compared in healthy volunteers the bioavailability after oral administration was 0.1%, using a 200 µg tablet (Fjellestad-Paulsen et al. 1993b). The absolute bioavailability of a direct application of 400 µg of an aqueous solution of dDAVP at different regions of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, colon and rectum) was studied in healthy volunteers (d'Agay-Abensour et al. 1993). Absorption from the ileum was lower (0.02%) than expected but the bioavailability of dDAVP after gastric, duodenal and jejunal application (0.2%) was similar to that obtained in the previous study. No preferential site of absorption was found, within the upper segment of the intestine, although two earlier animal studies had indicated a possible area of preferential absorption of dDAVP in the distal part of the ileum in rabbits (Lundin & Vilhardt 1986) and in rats (Lundin et al. 1991). In a recent study the stability of the neurohypophyseal hormones AVP and oxytocin, and of their synthetic analogues, was studied in human intestinal contents, the brush border membranes of the small intestine and in gastric, colonic and rectal plasma membranes (Fjellestad-Paulsen et al. 1995). dDAVP proteolysis was slower in jejunal than in ileal juice. The degradation was pH-dependent and a concentration dependent inhibition occurred when aprotinin was preincubated with contents from the ileum.

From a therapeutic point of view it would be of great importance to be able to increase the intestinal absorption of peptides that are pharmacologically active. One way would be to decrease the intestinal proteolysis. Several studies in animals have shown the feasibility of insulin absorption from the intestine in the presence of a protease inhibitor (Morishita et al. 1992). Aprotinin which is a single chain polypeptide consisting of 58 aminoacid residues is a potent inhibitor of several serine proteinases, such as trypsin, chymotrypsin, kallikrein and plasmin. A study in healthy volunteers showed that aprotinin injected together with insulin subcutaneously is followed by higher plasma levels of insulin than when insulin is injected alone (Owens et al. 1988). On the contrary, in nasal insulin absorption studies with the absorption enhancer sodium taurodihydrofusidate in rats and rabbits, co-administration of aprotinin did not alter significantly the absorption of insulin (Deurloo et al. 1989).

Similarly, no effect was seen on the antidiuretic effect when nasal administration of AVP and dDAVP were co-administered with aprotinin intranasally in rats (Morimoto et al 1991). Aprotinin has been administered in the duodenum (5 ml/min) in healthy volunteers (Dlugosz et al. 1983) without giving any side effects, and aprotinin is not destroyed by intestinal proteolysis (Frey et al. 1967) or absorbed by the intestinal mucosa (Markwardt 1978). Saffran et al. (1988) reported an increased oral activity of dDAVP in hydrated rats by simultaneous administration of an inhibitor of intestinal proteolysis.

In the present study, the $t_{1/2}$ of dDAVP after i.v. administration was 94 (12) min which is slightly more than in previous studies where a specific antiserum was used to measure plasma dDAVP (55, 78 and 60 min respectively) (Vilhardt et al. 1986, Fjellestad-Paulsen et al. 1993b, d'Agay-Abensour et al. 1993). The bioavailability of dDAVP after duodenal administration (0.09%) is similar to the bioavailabilities in our previous studies (Fjellestad-Paulsen et al. 1993b, d'Agay-Abensour 1993). Bioavailability of dDAVP together with a perfusion of aprotinin was however five times higher in the present study.

Aprotinin is a proteinase inhibitor, and thus it is not surprising that the lipase activity remained unchanged, while the chymotrypsin activity decreased significantly. These results are in accordance with those of a previous study (Dlugosz 1983).

In conclusion, the bioavailability of dDAVP after direct duodenal application of an aqueous solution was similar to that after swallowing a tablet. When given together with a perfusion of an enzyme inhibitor the bioavailability of dDAVP increased five times. It would be of great interest to enhance the efficacy of oral dDAVP administration by associating dDAVP with a protease inhibitor to avoid degradation by pancreatic enzymes. A drawback is the possible effect on digestion of dietary proteins by the co-administration with a protease inhibitor.

Acknowledgement

Dr Robert Chollet, Mrs Brigitte Huchet and Mrs Claire Franchisseur, INSERM U290 and Department of Gastroenterology, Hopital Saint Lazare, Paris, France are thanked for technical assistance. We thank Mrs Anja Broeders, Ferring Pharmaceuticals, Malmö, Sweden, for help with measurements of dDAVP in plasma and Ms Anette Persson for secretarial assistance.

Table 1. AUC (UI/240 min) of chymotrypsin and lipase activity during a 4 hours intestinal perfusion with (w) or without (wo) aprotinin in healthy volunteers (n = 6).

| Subjects | Chymotrypsin | | Lipase | |
|----------|--------------|--------|---------|---------|
| | w | wo | w | wo |
| BRAB | 5 948 | 19 573 | 403 680 | 336 690 |
| COPH | 8 469 | 18 479 | 614 790 | 353 670 |
| EMPL | 7 454 | 18 352 | 258 780 | 285 480 |
| JOMI | 5 510 | 22 678 | 334 440 | 264 720 |
| MARI | 1 795 | 10 501 | 135 150 | 200 340 |
| NIPL | 9 511 | 15 098 | 406 170 | 221 040 |
| x | 6 448 | 17 447 | 358 835 | 276 990 |
| SD | 2 730 | 4 184 | 161 497 | 61 110 |
| p | p < 0.001 | | NS | |

Table 2. Pharmacokinetic data for six male healthy volunteers receiving an i.v. bolus dose (4 µg) of dDAVP and after duodenal administration of 500 µg of dDAVP either with (W) or without aprotinin (WO) (CI = 95% confidence intervals).

| | IV | | INTESTINAL | | | | |
|----------------|-------------|------------------|------------------|--------------|--------------------|-------------|--------------------|
| | CL | t _{1/2} | AUC pg x h/ml | | F _{abs} % | | F _{rel} % |
| | ml/min | h | W | WO | W | WO | W |
| Subject | | | | | | | |
| BRAB | 68.8 | 1.28 | 317.3 | 106.1 | 0.33 | 0.11 | 299 |
| COPH | 67.7 | 1.79 | 278.5 | 45.4 | 0.28 | 0.05 | 613 |
| EMPL | 90.4 | 1.53 | 328.7 | - | 0.45 | - | - |
| JOMI | 90.8 | 1.77 | 238.1 | 36.9 | 0.32 | 0.05 | 645 |
| MARI | 87.1 | 1.51 | 643.3 | 105.3 | 0.84 | 0.14 | 611 |
| NIPL | 84.7 | 1.48 | 420.4 | 87.7 | 0.53 | 0.11 | 480 |
| \bar{x} (SD) | 81.6 (10.6) | 1.56 (0.20) | 371.1 (146.6) | 76.3 (33.0) | 0.46 (0.21) | 0.09 (0.04) | 529 (144) |
| CI | 70.5 - 92.7 | 1.36 - 1.76 | 217.2 - 524.9 | 35.3 - 117.3 | 0.24 - 0.68 | 0.04 - 0.14 | 351 - 708 |
| | | | | | | | |
| Paired | | | 0.0086 | | 0.0148 | | |
| T-test | | | | | | | |

Legends to figures

- Fig 1. Triple-lumen tube in position in the duodenum and the jejunum: localization of the inflatable balloon, perfusion and sampling sites are indicated.
- Fig 2. Mean intraluminal activity of chymotrypsin during a 4 hours duodenal perfusion with (■) or without (□) aprotinin after direct application of dDAVP in the duodenum in 6 healthy volunteers.
- Fig 3. Mean plasma concentrations (pg/ml) of dDAVP after an i.v. bolus injection of 4 µg of dDAVP (—), 500 µg dDAVP with aprotinin (— —) and 500 µg dDAVP alone (---) given in the duodenum in healthy volunteers (n = 6).

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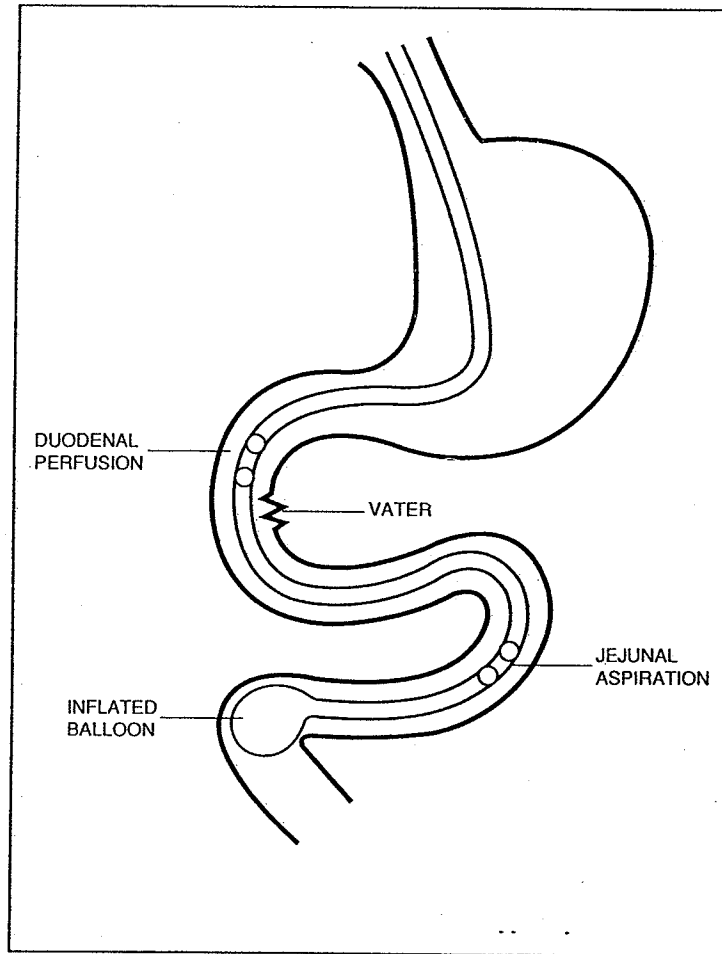


Fig. 1. Triple-lumen tube in position in the duodenum and the jejunum: localization of the inflatable balloon, perfusion and sampling sites are indicated.

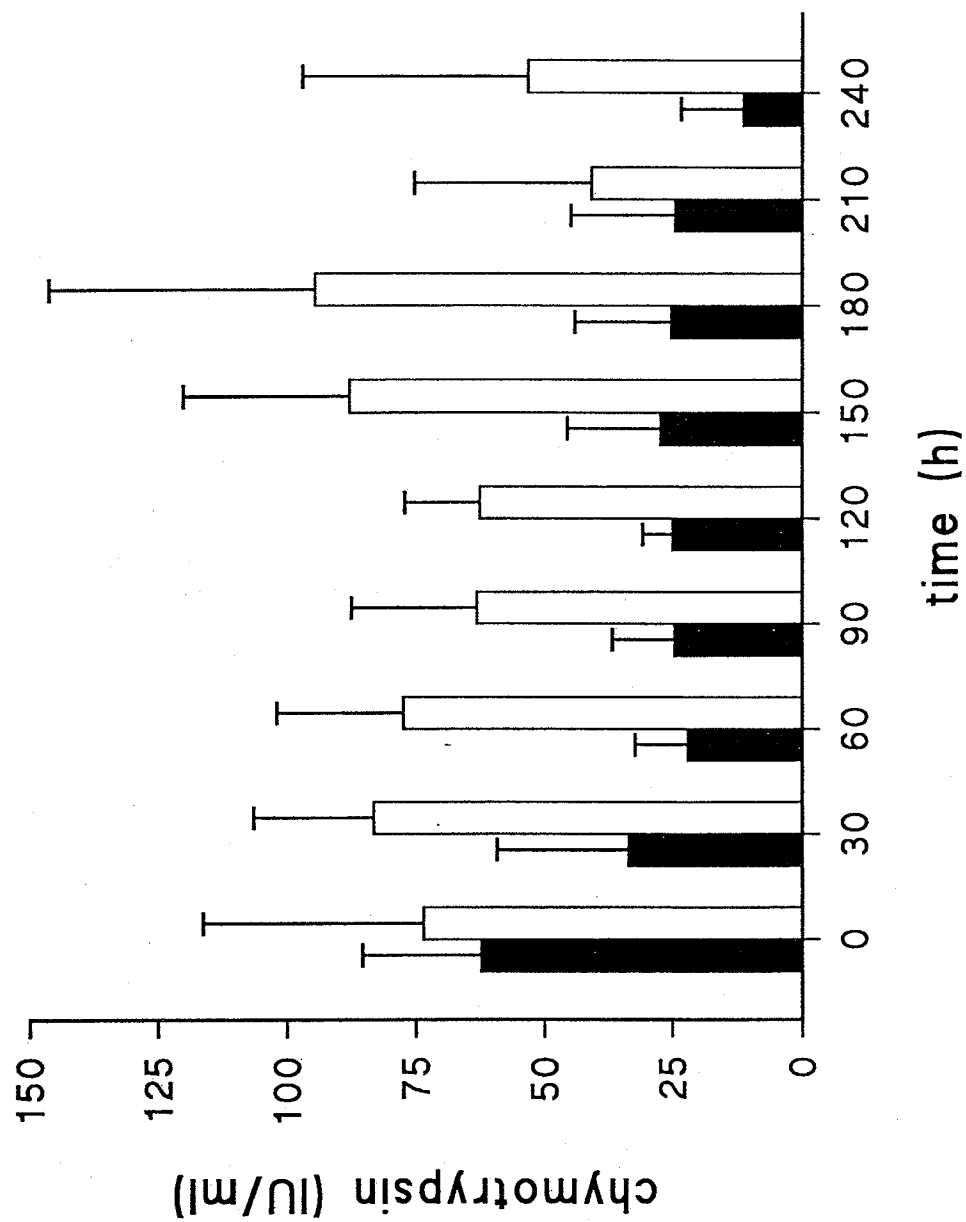


Fig. 2. Mean intraluminal activity of chymotrypsin during a 4 hours duodenal perfusion with (■) or without (□) aprotinin after direct application of dDAVP in the duodenum in 6 healthy volunteers.

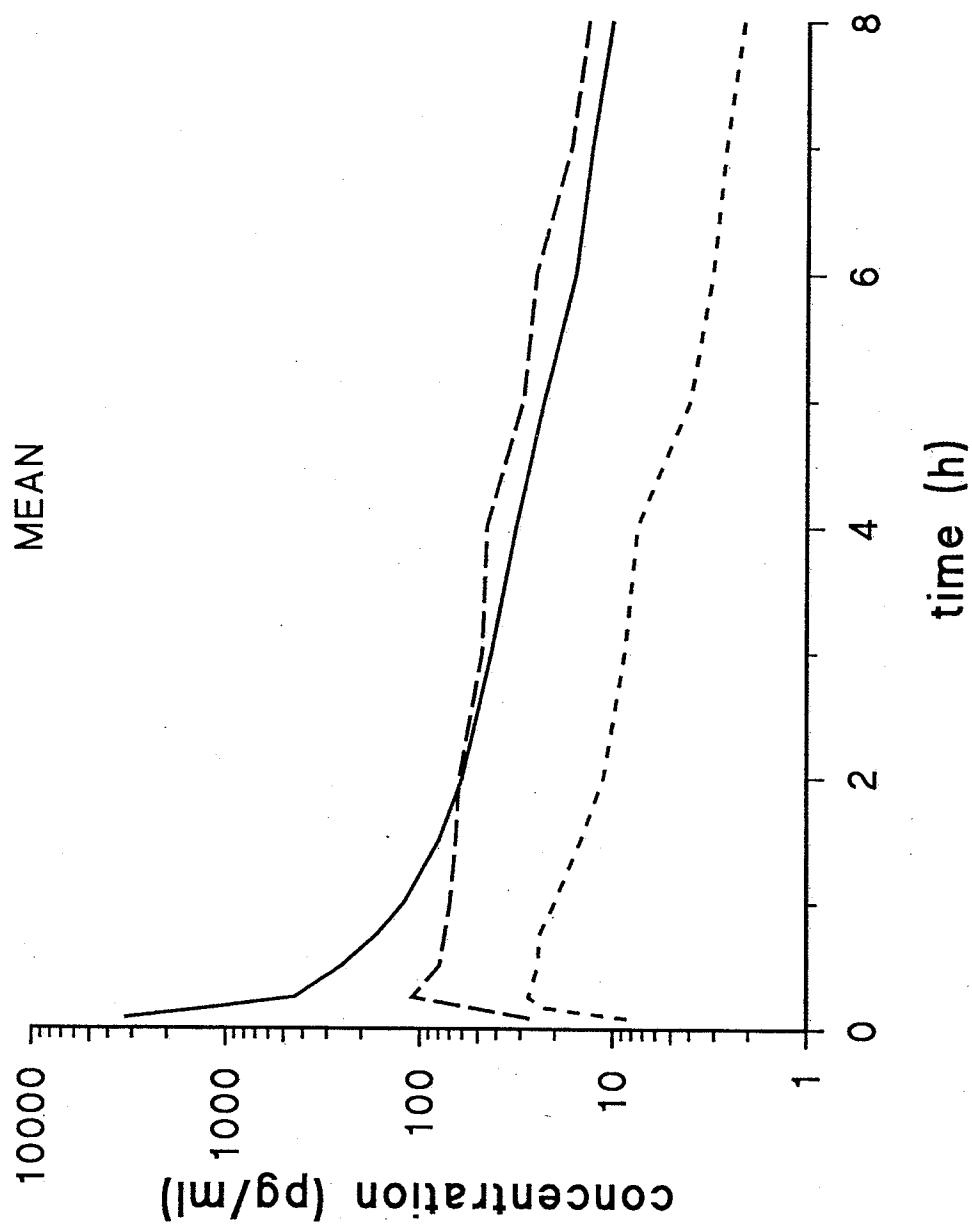


Fig. 3. Mean plasma concentrations (pg/ml) of dDAVP after an i.v. bolus injection of 4 μg dDAVP (—), 500 μg dDAVP with aprotinin (---) and 500 μg dDAVP alone (-.-) given in the duodenum in healthy volunteers (n=6).

VI

Central diabetes insipidus in children. Antidiuretic effect and pharmacokinetics of intranasal and peroral 1-deamino-8-D-arginine vasopressin

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Abstract. The antidiuretic effect and pharmacokinetics of 10 to 20 µg of intranasal (IN) and 200 to 400 µg of oral (po) 1-deamino-8-D-arginine vasopressin (DDAVP) were studied in 10 paediatric diabetes insipidus patients. A significant increase in urine osmolality was obtained with all doses, maximum within 2 h and still present at 8 h. At 12 h after administration, the ratio urine osmolality/plasma osmolality was above 1 only after 20 µg intranasally and 400 µg perorally. The free water clearance decreased rapidly with all doses and was similar in magnitude and duration for both the intranasal and peroral routes of administration and remained negative for more than 8 h. The maximum plasma concentrations of DDAVP, measured with a specific and sensitive RIA method, was dose-dependent and there was not significant difference in time until maximum concentration was obtained or in plasma half-life between the two routes of administration. The ratio established, 1:20, by calculating the area under the curve showed a bio-equivalence between 10 µg IN and 200 µg po and between 20 µg IN and 400 µg po of DDAVP. This work further emphasized the effectiveness of the oral route and the rapidity of absorption. By continuous monitoring of DDAVP plasma values we have demonstrated that peak values were reached within one hour after administration. This study demonstrates that the doses needed to treat diabetes insipidus patients by the oral route will be approximately 20 times greater than by the nasal route.

For more than 15 years, patients suffering from neurogenic diabetes insipidus (DI) have been treated with intranasal (IN) 1-deamino-8-D-argin-

ine vasopressin or DDAVP (Andersson & Arner 1972; Becker & Foley 1978), a synthetic analogue of arginine vasopressin (AVP). Besides the most common mode of administration, intranasal, (Aronson et al. 1973; Robinson 1976) other routes have been used successfully including intravenous, intramuscular, subcutaneous and sublingual administration (Edwards et al. 1973; Grossman et al. 1980; Laczi et al. 1980). Recently oral (po) treatment has been described as an attractive alternative to IN administered DDAVP (Vilhardt et al. 1985). In a recent study we have demonstrated the feasibility and effectiveness of treatment of DI in children by the oral route (Fjellestad & Czernichow 1986). This has been confirmed by other authors (Westgren et al. 1986). The purpose of the present study was to determine the equivalent IN and po doses needed to obtain a similar biological effect. A study was therefore designed to compare the effect and plasma DDAVP profiles obtained after 10 and 20 µg of DDAVP, IN and after 200 and 400 µg DDAVP, po, in 10 paediatric DI patients.

Materials and Methods

Patients

Informed consent was obtained from all parents or adolescents and the protocol was approved by the

hospital ethical committee. Seven boys and three girls aged from 4.5 to 19 years (mean 9.4) had central DI of varying etiology: histiocytosis (N = 3), craniopharyngioma (N = 2), hypothalamic dysgerminoma (N = 1), and isolated idiopathic DI (N = 4). All children had previously been treated with IN DDAVP and 6 of them had been controlled successfully with po DDAVP for more than 18 months without any adverse reactions. When necessary, anterior pituitary replacement therapy was maintained during the study.

DDAVP

DDAVP was provided by Ferring Pharmaceuticals, Malmö, Sweden. The IN solution was identical to the commercially available preparation (Minirin®, 100 mg/l) and tablets were provided containing 200 µg of DDAVP per tablet.

Protocol

Treatment with IN or po DDAVP was discontinued for 36 h prior to the investigation. The patients were hospitalized for 5 days and they all received the different doses in the same order. Day 1 and 2: 10 and 20 µg of IN DDAVP and day 4 and 5: 200 and 400 µg tablets, respectively. The children did not receive any evening dose and were without any treatment on day 3. All had a normal breakfast and fluid intake ad libitum was permitted throughout the study. DDAVP was administered at the same time each morning and blood was

collected at regular intervals for 12 h from a butterfly needle in a forearm vein. Urine volume, plasma (P osM) and urine (U osM) osmolality, and serum sodium were determined every hour for 2 h and every 2 h up to 12 h.

Laboratory methods

U osM and P osM were measured by an Advanced Osmometer. Blood (5 ml) was collected in heparinized tubes and plasma stored at -20°C. Plasma-DDAVP was measured by RIA (Lundin et al. 1985) using an antiserum specific for DDAVP, raised in Dutch rabbits against 8-D-arginine vasopressin. DDAVP was extracted from plasma (Robertson et al. 1973).

Statistical analysis: Comparison between groups was assessed with Student's paired *t*-test.

Results

Antidiuretic effect

The antidiuretic response following different doses of DDAVP is shown in Fig. 1 and Table 1. The U osM increased rapidly for all doses during the second hour and in a similar way after IN and po administration. Up to 8 h after administration there was no difference between the 4 doses. At 8 h, the difference between 10 and 20 µg of IN

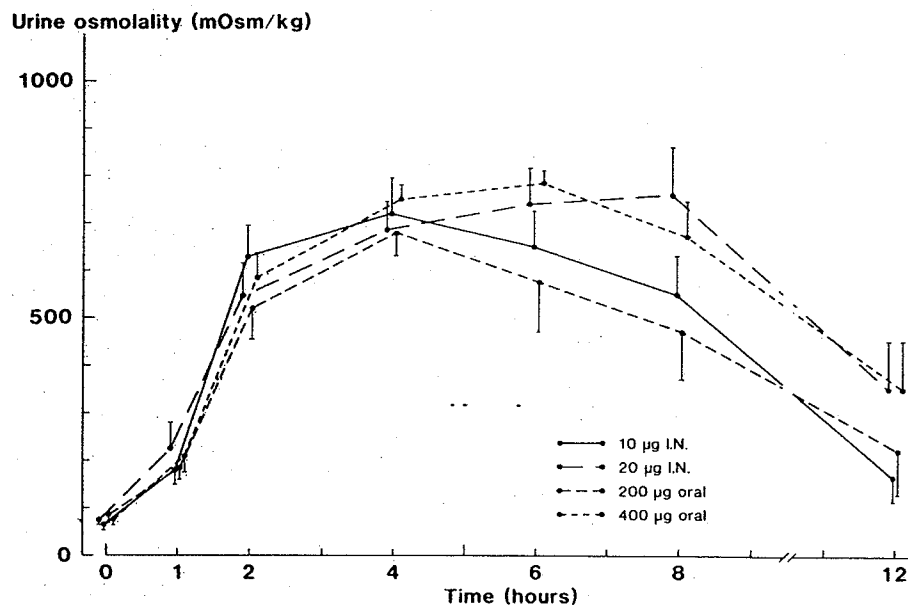


Fig. 1.

Urine osmolality (mosmol/kg, mean \pm SEM) in 10 DI patients following intranasal DDAVP (10 µg ●—●; 20 µg ●—●), and oral DDAVP (200 µg ●---●; 400 µg ●---●). IN: intranasal.

Table 1.
Urine osmolality (mosmol/kg, mean \pm SD) following intranasal and oral DDAVP 2, 8 and 12 h after administration and U osM maximum values.

| | Urine osmolality (mOsm/kg) | | | |
|----------------|----------------------------|---------------|---------------|---------------|
| | Mean \pm SD | | | Max. \pm SD |
| | 2 h | 8 h | 12 h | |
| 10 μ g IN | 630 \pm 190 | 555 \pm 237 | 167 \pm 158 | 756 \pm 201 |
| 20 μ g IN | 540 \pm 226 | 761 \pm 282 | 349 \pm 319 | 828 \pm 198 |
| 200 μ g po | 530 \pm 150 | 468 \pm 319 | 221 \pm 283 | 733 \pm 156 |
| 400 μ g po | 585 \pm 140 | 671 \pm 242 | 352 \pm 313 | 809 \pm 77 |

DDAVP was significant ($P < 0.01$). The maximum U osM was higher after the largest doses tested (20 μ g IN and 400 μ g po), but the difference was not significant. A difference was observed in the duration of effect. After 12 h the mean U osM was still above 350 mosmol/kg with 20 μ g of DDAVP IN and 400 μ g po, whereas with 10 μ g IN and 200 μ g po the U osM was 167 and 221 mosmol/kg, respectively. P osM remained stable during 12 h for the 4 doses. Free water clearance (FWC) showed no difference between doses or routes of

administration of DDAVP and remained negative for 7 to 8 h. After administration of DDAVP, the mean FWC fell from 2.89 l/min to -0.24 after 2 h, was -0.39 after 8 h, and increased to $+0.16$ after 12 h. The mean (\pm SD) duration of U osM remaining above 400 mosmol/kg was 7.4 h \pm 3.0 and 9.0 h \pm 3.2 after 10 and 20 μ g of IN DDAVP and 7.2 h \pm 3.3 and 8.8 h \pm 2.3 after 200 and 400 μ g po, respectively. A statistical difference was found only between 10 and 20 μ g of in DDAVP ($P < 0.05$).

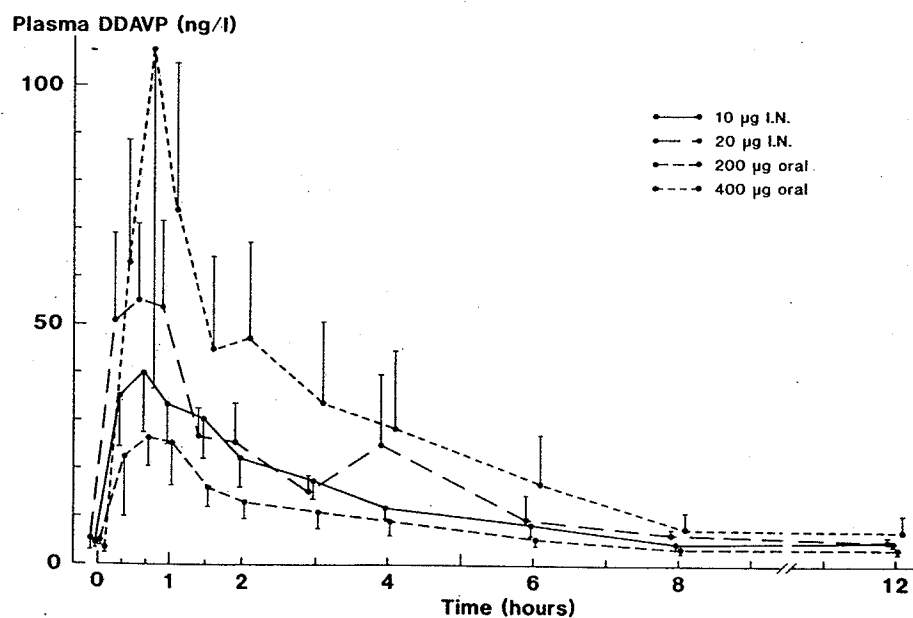


Fig. 2.
Plasma DDAVP concentration (ng/l, mean \pm SEM) following intranasal DDAVP (10 and 20 μ g) and oral DDAVP (200 and 400 μ g) in 10 children with diabetes insipidus. IN: intranasal.

Table 2.

Plasma DDAVP: Pharmacokinetics of DDAVP in 10 DI patients following 10–20 µg of intranasal DDAVP and 200–400 µg of oral DDAVP (mean ± SD). Area under the curve, maximum concentration (plasma value and time in min to reach this value after administration of the drug), and plasma half-life are indicated.

| Dosage (µg) | Area under the curve (ng/l × h) | Maximum concentration | | Plasma half-life T (h) |
|----------------|---------------------------------------|-----------------------|---------|------------------------------|
| | | ng/l | T (min) | |
| Intranasal | | | | |
| 10 µg IN | 156.6 ± 109.3 | 41.4 ± 31.6 | 41 ± 23 | 3.26 ± 0.50 |
| 20 µg IN | 239.7 ± 202.1 | 75.6 ± 70.3 | 39 ± 14 | 3.80 ± 0.71 |
| Oral | | | | |
| 200 µg | 121.6 ± 67.1 | 33.2 ± 30.7 | 48 ± 21 | 3.97 ± 1.82 |
| 400 µg | 260.2 ± 379.5 | 103.9 ± 176.4 | 49 ± 19 | 2.64 ± 1.06 |

Pharmacokinetics of DDAVP

The plasma concentrations of DDAVP after IN and po administration of DDAVP are shown in Fig. 2 and Table 2. DDAVP appeared in the plasma already 20 min after administration. Maximum concentration (see Table 2) was obtained for the different doses after 39 to 50 min and thereafter the concentration decreased following first order kinetics for both the IN and po routes of administration. The calculated plasma β -half-life of DDAVP ranged between 3.3 and 4.0 h and was not different after po or IN dosing. The area under the curve (AUC) for the plasma concentration of DDAVP (Table 2) showed a significant difference between the two groups 10 µg IN/200 µg po and 20 µg IN/400 µg po ($P < 0.05$).

Discussion

From the first description of the biological effect of DDAVP in humans, the clinical value of DDAVP in DI has been emphasized in several papers. However, DDAVP is usually administered intranasally and physicians are aware of the difficulties of controlling polyuria when, for some reason, the nasal route is not available. It has been proved recently that AVP, 1-d-AVP, and especially DDAVP have a biological activity when taken orally. This is clinically valuable, especially in patients with defective eyesight, frequent rhinitis, and in small children. A dose-dependent antidiu-

retic response after intragastric or oral administration of DDAVP has been shown in hydrated dogs, normal humans, and adults suffering from DI (Vilhardt & Bie 1983, 1984; Hammer & Vilhardt 1985). After intragastric administration of DDAVP in rats, the plasma concentration of DDAVP confirms the gastrointestinal passage of this nonapeptide (Lundin et al. 1985). In a recent study, Fjellestad & Czernichow (1986) showed a dose response relationship of oral DDAVP in 10 children with DI. The same children have been treated orally for more than 18 months, without any adverse reactions and showing no relapse in effect. As with the IN route, the po doses necessary presented large individual differences, but were higher in total dosage (100 to 300 µg 2 to 3 times daily). Plasma concentrations of DDAVP measured after an iv bolus injection in patients with DI showed the plasma half-life to be 51 min (Edwards et al. 1973) and 158 min for the α and β phases, respectively (Pullan et al. 1978), and after IN administration the plasma half-life varied from 0.4 to 4 h (Seif et al. 1978). In these studies there was no correlation between plasma half-life and duration of antidiuretic effect, suggesting that the inter-individual differences observed in duration are related to the absorption. The anti-serum used in these studies for the measurement of DDAVP was raised in a non-specific way against vasopressin, and direct comparison with the results of the present study is therefore difficult. Using a more specific RIA, Vilhardt et al. (1986) found a plasma half-life of 55 min after iv administration in humans.

In Brattleboro rats, Lundin et al. (1985) showed a dose-dependent increase in the plasma concentration of DDAVP after intragastric administration, still measurable after 6.5, but undetectable after 20 h. In the study by Hammer & Vilhardt (1985), the plasma concentrations after an oral dose of DDAVP increasing from 12.5 to 400 µg in 4 adult DI patients, showed a dose-dependent increase and the calculated plasma half-life varied from 1.2 to 3.6 h.

In our study, the plasma concentrations of DDAVP showed a peak which appeared early, and the plasma levels were still detectable after 8 h, suggesting a rapid transmucosal absorption from the gastrointestinal tract. The persistence of DDAVP in the plasma is probably due to slow elimination of the drug. However, according to the shorter half-life reported after iv administration, the persistence of DDAVP in plasma after po administration may also be due to a prolonged absorption phase of the peptide.

There was no significant difference in time till maximum concentration or in plasma half-life between the IN and po routes, and the maximal plasma concentration was dose-dependent. With regard to the AUC, the difference between the IN and the po route was significant in the two groups, 10/200 µg and 20/400 µg ($P < 0.05$) showing a bio-equivalence between these two modes of administration.

The IN doses necessary vary in several studies and are not found to be related to age, the severity of polyuria, or to the body weight of the patient (Aronson et al. 1973). In individual patients the antidiuretic effect may vary from 6 to 24 h after IN administration of 20 µg (Robinson 1976) or from 5 to 21 h (Seif et al. 1978). In the present study, the individual variations were large too, as illustrated by the plasma DDAVP values. One patient had very low plasma DDAVP after po administration and needed high doses to maintain an acceptable antidiuresis, whereas this patient's in doses were not different from the mean doses. A different metabolism and/or clearance of DDAVP in some patients will necessitate an individual adjustment of the po regimen.

Our conclusions from this study are that there is a relationship between dose and effect for the po route and that the ratio between the IN and po dose is 1:20. Even if the maximum U osM obtained with the IN and po doses was similar, it is important to consider the duration of effect after

12 h. Therefore, we recommend doses of 400 µg two times or 200 µg three times daily for oral treatment. It is necessary, especially in children, to increase the evening dose to assure a satisfactory antidiuresis during sleep.

Acknowledgments

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Errata

- page 309, right column, line 8: "mosmOL/kg" read "mosMol/kg"

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LUND

ANNE M. KJELLESJAU-PULSEN

ADSPHON AND THE GROUNDWATER OF THE PLYMOUTH COAST

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PATENT
Attorney Docket No. SER-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | | |
|-----------------|--|-------------------------|------|
| APPLICANT(S): | Seymour Fein | GROUP NUMBER: | 1614 |
| | | CONFIRMATION NUMBER: | 7710 |
| APPLICATION NO: | 10/706,100 | EXAMINER: | Tate |
| FILING DATE: | November 12, 2003 | | |
| TITLE: | PHARMACEUTICAL COMPOSITIONS INCLUDING LOW DOSAGES OF DESMOPRESSIN | | |

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF RONALD V. NARDI UNDER 37 CFR §1.132

Sir:

I, Ronald V. Nardi, declare as follows:

1. I have read the above-identified patent application, including the claims currently pending, the official action mailed March 14, 2007 finally rejecting the claims, and the references applied therein as a basis of the rejection. My *curriculum vitae* is attached as Exhibit A. In relevant summary, I hold a Ph.D. in Pharmacology/Toxicology; I have spent essentially my entire career working in companies in the business of developing drug formulations; and, I am very experienced in and familiar with the pharmaceutical properties of desmopressin. Among other positions, I worked from 1996 until 2002 with Ferring Pharmaceuticals, Inc. and Ferring Group Research and Development, which markets desmopressin for various urological pharmaceutical uses under the brand name Minirin®.

2. The above-referenced Fein application has been assigned to Reprise Pharmaceuticals, LLC, and all rights therein have been licensed exclusively world-wide by Reprise to Serenity Pharmaceuticals Corporation. Reprise holds an equity position and contractual right to royalties under any patent issued from the above-referenced application. I serve as a paid consultant to the licensee Serenity and hold an equity position in Reprise, through which I hope to benefit should the above-referenced application issue as a patent and a product covered by the claims thereof be marketed in the United States.

3. At an interview in the Patent Office on April 26, 2007, I understood Examiner Tate to question whether a person of skill in the drug formulation art is able to make dosage forms falling within the claims. As a person of skill in this art, I can state without reservation that skilled persons were able, at the time the application was filed, to make various dosage forms adapted for intranasal, transmucosal, transdermal, conjunctival, or intradermal administration that will maintain in a patient a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0 picograms desmopressin per ml plasma/serum. Given the clearance rate of desmopressin (on the order of a 1.5 to 2.5 hour half-life in humans), this could be done simply by reducing the amount and/or concentration of active in a selected dosage form appropriately. Over the last 30 years the industry has developed sophisticated transmucosal drug delivery technology including intranasal, buccal, and sublingual dosage forms (as described in the specification of the Fein application), as well as intradermal and transdermal dosage forms. These provide a large number of options for the preparation of low dose desmopressin dosage forms having the properties set forth in the claims.

4. I understand the claims stand rejected as being anticipated, that is, unpatentable because embodiments of the claimed compositions allegedly have been identically disclosed, by U.S. Patent Nos. 5,707,648 (“Yiv”), 6,693,082 (“Alonso”), 6,746,768 (“Shapiro”), and 4,863,737 (“Stanley et al.”), and by Trinh-Trang Tan et al., (*J. Am. Soc. Nephrol.*, 2000, Meeting Abstract), Wolfson et al. (*Am. J. Gastroenterol.*, 1979), Jahr et al. (*Anesthesia & Analgesia*, 1992), Dixon et al. (*Br. J. Radiol.*, 1981), Malan et al. (*Toxicol. Methods*, 1994), or Tormey et al. (*Eur. J. Internal Medicine*, 1992).

5. The Examiner states in his official action that: (1) the various desmopressin pharmaceutical compositions disclosed in the references are

“adapted so as to be suitable for being added to an intranasal, transmucosal, transdermal, conjunctival, and or intradermal formulation/patch”

and (2) that administration of the desmopressin pharmaceutical compositions disclosed in each of these references would

“inherently provide the instantly claimed functional effect upon administration” in that, if the desmopressin formulations taught by the references were administered in a proper form, “a steady plasma/serum desmopressin concentration within the approximate instantly claimed range, as well as a decrease in urine production, would inherently occur.”

6. All of the pending claims require a pharmaceutical composition:

in a dosage form adapted for intranasal, transmucosal, transdermal, conjunctival, or intradermal administration

sufficient to establish in a patient

a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0 picograms desmopressin per ml plasma/serum and

to decrease urine production.

To a person of skill in the art, as the Applicant previously has noted, this means that the dosage forms claimed must establish a very low concentration of desmopressin in the patient's circulatory system *which persists for some significant period of time at one or various concentrations within the recited low serum concentration range* so that urine production is decreased for some constant and predictable period of time, *e.g.*, 2-8 hours, or 4-6 hours.

7. The dosage forms possessing the features recited in the claims achieve a novel and surprising effect, as they can effectively interrupt urine production - that is, induce voiding postponement, less frequent urination, and other antidiuretic effects, yet avoid, decrease or eliminate induction of hyponatremia. This is accomplished by controlling the duration of the anti-diuretic effect of desmopressin by controlling its blood concentration and switching it “off”

at the desired time as the concentration of the circulating drug is cleared by the body and falls below a concentration effective to activate kidney water channels.

8. My review of the references suggests that none of them disclose, either expressly or inherently, any dosage form having the combination of features set forth in the claims. As set forth below in more detail, the Examiner is correct that some of the references disclose dosage forms that fairly can be said to be adapted for intranasal, transmucosal, intradermal, or transdermal administration. However, none inherently achieve, or expressly suggest achieving, “a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0 picograms desmopressin per ml plasma/serum ...”

9. The prior art does disclose that desmopressin may be administered intranasally (*see* Shapiro; also, note that there is an FDA approved, intranasally administered desmopressin product on the market in the United States, *see* Focus on Urological Indications, Minirin® desmopressin, attached as exhibit B, hereinafter referred to as the “Monograph”), transmucosally (*see* Stanley et al., which discloses a “lollipop” formulation for “absorption through the mucosal tissues of the mouth, pharynx, and esophagus”), transdermally (*see*, U.S. Patent No. 4,878,892 to Sabalis et al., which discloses a device for transdermal transport of polypeptide such as desmopressin to the bloodstream of the patient), and intradermally (*see* U.S. Patent No. 5,841,991 to Gross et al., which discloses intradermal drug delivery devices for delivering a liquid drug such as desmopressin to a subject via the subject’s skin). However, none of the cited or applied references, and no reference known to me, teach any desmopressin dosage form which establishes a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0 picograms desmopressin per ml plasma/serum and meets the other requirements of the claims.

10. The bioavailability of a drug is defined as the amount of drug taken or administered that actually reaches the circulation and therefore can have a physiological effect. For desmopressin, which is a peptide hormone analog, oral, transmucosal, and transdermal bioavailability is poor because only a small fraction of the drug administered is able to reach a patient’s bloodstream through the gastrointestinal tract, across mucosal tissues of the mouth or nasal passages, or

through the skin. I believe the currently accepted oral bioavailability of desmopressin is about 0.08% to 0.12% (*see* Monograph, page 5, Table 2). This bioavailability is low because molecules of the size of desmopressin are poorly absorbed and because peptides are inactivated by digestion in the stomach and the completeness of the digestion varies, influenced by many factors, including diet. Intranasal bioavailability of the currently available desmopressin product is about 3% to 4%, (*see* Monograph, page 5, Table 2). Oral transmucosal bioavailability of desmopressin is in the range of 0.25%. This range is low and very broad as the oral mucosal (buccal) surface area and permeability vary among individuals, and because the dwell time of any dosage form in position adjacent the membranes in the mouth varies widely, with unknown amounts of the active in conventional buccal dosage forms being diluted in saliva and swallowed, and essentially lost by digestion in the stomach. Transdermal bioavailability is dependent on many formulation factors known to the skilled artisan. Dosage forms adapted for parenteral delivery, including those adapted for subcutaneous, intramuscular, intra-arterial, and intravenous delivery modalities have a bioavailability of essentially 100%.

11. When bioavailability is taken into account, it is apparent that none of the cited references disclose an intranasal, transmucosal, transdermal, conjunctival, or intradermal dosage form that inherently achieves a steady plasma/serum desmopressin concentration in the range required by the claims. All of the dosages I have seen described in the applied art result in a serum concentration in excess of - and typically far in excess of - the concentration range recited in the claims. While it is possible to formulate low dosage forms if one sets out to achieve such a sustained low concentration, none of the cited references make any such attempt. The reason for this perhaps is that, at such low serum concentrations, desmopressin has been thought to be ineffective to interrupt urine production significantly. An example of this belief may be appreciated from a reading of Dixon et al. (*Br. J. Radiol.*, 1981), cited by the Examiner. Dixon et al. disclose that a 200 ng IV dose of desmopressin (approximately 3 ng/kg for 70 kg person, essentially 100% bioavailable, producing a maximum desmopressin blood concentration *greater than 10 pg/ml*) *does not produce a significant change in urine flow rates* compared to saline controls (*see* page 642, “*Effect of DDAVP on urine flow rate*”). The authors chose, based on their studies, to evaluate 1 µg and 4 µg IV doses of desmopressin, both of which produced statistically significant reduction in urine flow rates and blood concentrations far in excess of that set forth in the claims. These data suggest that urine production is not affected by exogenous

desmopressin at about 3 ng/kg (>10 pg/ml) but can be reduced with 1 µg doses (approx. 14 ng/kg and higher). In total, these data would suggest to me that doses having the properties required by the claims would be *ineffective* to interrupt urine production.

12. U.S. Patent No. 5,707,648 to Yiv discloses a capsule “for oral, rectal, and vaginal, preferably oral and rectal, and more preferably oral,” administration, with the lowest suggested dose being “13 micrograms of desmopressin for administration to dogs weighing from 9 to 12 kg.” This means that, at the known percent bioavailability of transmucosal or oral doses of desmopressin, and assuming the entire lowest dose is absorbed in 90 minutes, the serum concentration will be between about 50 and 150 pg/ml. But Yiv’s purpose is to *increase* oral, rectal and vaginal bioavailability of desmopressin, thus to increase serum plasma concentration over that conventionally achieved with such dosage forms. The lowest amount of desmopressin suggested for use by Yiv that I could find is 4 µg in a *subcutaneous* dosage form for administration to 9-12 kg dogs. This dosage form would have approximately 100% bioavailability in the dog and would result in a serum concentration of about two orders of magnitude higher than the highest serum concentration delivered by the dosage forms claimed in this application.

13. U.S. Patent No. 6,693,082 to Alonso et al. discloses *intravenous* dosage forms of desmopressin. However, there is no literal or inherent teaching in the reference that any desmopressin dosage form disclosed can or should establish a steady desmopressin plasma/serum levels between 0.1 and 10.0 picograms/ml. The lowest dosage of desmopressin I could find in Alonso is 0.3 µg/kg of body weight which, for a 70 kg person, would mean about a 20 µg dose. However, the only dosage forms taught by Alonso appear to be *intravenous*, and thus characterized by 100% bioavailability. This means that plasma concentration (for a 10 minute infusion) would be about two orders of magnitude higher than the highest serum concentration delivered by the dosage forms claimed in this application.

14. U.S. Patent No. 6,746,678 to Shapiro discloses nasal administration of desmopressin in daily dosages ranging from 10 to 40 micrograms. However, these doses are conventional, and do not literally disclose or inherently establish a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0

picograms desmopressin per ml. A single 10 µg intranasal dose, at the lowest estimate of the range of intranasal bioavailability, in some individuals, would introduce into the blood a bolus of 300 to 400 ng of desmopressin, which when distributed would result in a desmopressin concentration of about 20-27 pg/ml, *i.e.*, about double or more than the largest dose claimed. In reality, the marketed DDAVP intranasal dosage form is labeled for administration of 10 µg *in each nostril*, leading to at least about double this serum concentration.

15. U.S. Patent No. 4,863,737 to Stanley et al. discloses a candy matrix for transmucosal delivery through the mucous membranes of the mouth, pharynx, and esophagus of drugs, including a long list of actives, and desmopressin, which can be present in amounts ranging from 10 to 50 micrograms. During use of any such dosage form, saliva production and swallowing will vary greatly among patients, varying amounts of the active will be transported to the gut (essentially never reaching the blood stream), and some unknown amount will be transported transmucosally into the circulation as Stanley et al. suggest. As such, this reference does not literally or inherently disclose establishment of a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0 picograms desmopressin per ml. Stanley et al. contains no data or facts upon which one could know what level of desmopressin, if any, would or could be achieved by such dosage form in any individual.

16. Trinh-Trang Tan et al., (*J. Am. Soc. Nephrol.*, 2000, Meeting Abstract), Wolfson et al. (*Am. J. Gastroenterol.*, 1979) Jahr et al. (*Anesth. Analg.*, 1992), Dixon et al. (*Br. J. Radiol.*, 1981), Malan et al. (*Toxicol. Methods*, 1994) and Tormey et al. (*Eur. J. Intern. Med.*, 1992) disclose dosage forms adapted for *intravenous, intra-arterial, subcutaneous, or intramuscular* administration of desmopressin. One cannot take a dosage form intended for such direct administration to the circulation and use it in very different intranasal, transmucosal, transdermal, conjunctival, or intradermal administration without significant modification. Accordingly, none of these references disclose a dosage form “adapted for intranasal, transmucosal, transdermal, conjunctival, or intradermal administration” as required by all claims in this application. Furthermore, all of the concentrations observed to have an anti-diuretic effect are disclosed to be present in the bloodstream of the patients or test animal involved at much higher blood concentrations than that required by the claims.

17. In conclusion, the Patent Office's position, that administration of the desmopressin dosage forms disclosed in the cited references inherently would establish in a patient a steady plasma/serum desmopressin concentration in the range of from about 0.1 picograms desmopressin per ml plasma/serum to about 10.0 picograms desmopressin per ml plasma/serum, appears to be incorrect. I was unable to find any disclosure in any of the cited references of any intranasal, transmucosal, transdermal, conjunctival, or intradermal dosage form which inherently establishes desmopressin plasma/serum levels within the recited range.

18. I could find no disclosure in any of the cited references that suggests that any blood concentration or dosage form of desmopressin is capable of safely interrupting urine production, enabling voiding postponement, or reducing the frequency or time between urination voiding, without substantial risk of dangerous side effects such as risk of developing hyponatremia, or that desmopressin blood concentrations in the range required by the claims necessarily is achieved or should be achieved by any of the dosage forms disclosed for any reason. None of the dosage forms in the cited references could produce or credibly claim to produce and maintain blood levels of desmopressin within the instantly claimed range so as to consistently and predictably control the duration of anti diuretic effect by controlling the timing of the pharmacological "off" mechanism.

19. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date:

May 11, 2007



Ronald V. Nardi

Exhibit A

RONALD VINCENT NARDI, Ph.D.

32 Hutton Drive
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BACKGROUND SUMMARY

Research and Development Executive broadly experienced in Drug Discovery and Development, Regulatory Affairs and Manufacturing.

- Doctoral degree in pharmacology/toxicology
- 28 years experience in drug discovery/development
- Supervision of R&D management, scientific personnel, drug discovery/development programs and budgets
- Responsibility for 26 INDs, 8 NDAs, 8 new pharmaceutical product approvals (6 since 1996) and 3 new device approvals
- Corporate management experience including strategic direction assessments, development of business plans and integration of commercial and R&D goals
- Involvement and contributions in all aspects including:
 - identification of new therapeutic targets
 - discovery of novel NCEs
 - preclinical and clinical research and development
 - formulation and analytical methods development
 - project and program management
 - regulatory affairs including IND and NDA submissions and approvals and interactions with regulatory agencies
 - manufacturing: operations and process development
 - medical/marketing interface
 - business development including acquisitions of technology and products, out-licensing and corporate partnerships
- Scientific articles in protein chemistry, pharmacology, cardiovascular diseases, gastrointestinal diseases, infertility, endocrinology, drug development, growth factors, and R&D management
- Patents on diagnostic and therapeutic innovations
- Experience with biotech industry (turn around and start-up situations) including preparation of business plan, financial management and venture fund raising, recruiting management and scientific staff and planning of personnel and facilities growth;
- In multiple small company situations, built substantial new product and NCE portfolios with global commercial potential in excess of \$2.0B.

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EDUCATION

B.A. 1970 Biology, Temple University,

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BioValve Technologies Inc.

Westborough, Massachusetts and Ramsey, New Jersey

Responsibilities

- Pharmaceutical and Technology Research and Development
- Regulatory Affairs
- Portfolio management
- Project management
- R&D budget management

Selected Accomplishments

- Regulatory approvals for 3 medical devices used to deliver novel therapeutics.
- Developed the therapeutic product portfolio to enable transition of the company from a technology development organization to a specialty pharmaceutical company with drug/device and drug only product opportunities.
- Identified and facilitated acquisition of development stage pharmaceutical company with a portfolio of pharmaceutical product opportunities.
- Intellectual property discoveries and inventions leading to patent application for novel devices (1) and NCEs (3 series of dopamine receptor agonists and antagonists)
- Established and prioritized research stage through clinical development stage portfolio of therapeutic products with global commercial potential in excess of \$3.0B.
- Provide the scientific support to the capital-raising activities of the company including technology licensing deals and equity investments.

Senior Vice President & Chief Scientific Officer

2003-2004

PDI Inc.

Research and Development

Upper Saddle River, New Jersey

Corporate responsibilities

- Corporate Research and Development

- Worldwide Regulatory Affairs
- R&D Portfolio management
- R&D business development
- Project management
- R&D budget management

Chief Scientific Officer & Executive Vice President

Ferring Group Research and Development
Ferring Pharmaceuticals SA, Switzerland
Ferring International Center A/S, Denmark

2001-2002

Corporate responsibilities

- Corporate Discovery Research
- Corporate Clinical Research and Development
- Pharmaceutical Research and Development
- Non-clinical safety and Toxicology
- Worldwide Regulatory Affairs
- R&D Portfolio management
- R&D business development
- International project management
- R&D budget management (\$70M+; 300+scientific staff)

Selected Accomplishments

- Approval of company's first global development product.
- INDs approved for 2 NCEs, CTXs approved for 3 NCEs
- Revised development program for highest priority NCE to increase market potential and cut development timeline
- Developed patent-protected line extension strategy for company's largest product
- Established a global drug development operation and organization
- Improved the medical/marketing interface and established a collaborative interaction between corporate R&D and the Operating companies
- Expanded drug discovery research activities to build a new product pipeline with market potential of \$2.0B
- Acquired technology to improve drug discovery operation
- Acquired two research stage product opportunities
- Established three development partnerships based on Ferring intellectual property

Vice President, Scientific and Regulatory Affairs

Ferring Pharmaceuticals, Inc., Tarrytown, New York

1996-2001

Selected Accomplishments

- FDA Approvals for five new products; supplemental NDA approvals affecting four products in the portfolio.
- Established functional departmental structure. Recruited staff for drug development, regulatory affairs, and manufacturing capabilities in the US.

- Established a cooperative medical/marketing interface to facilitate data oriented marketing of company products
- Established liaison with corporate drug discovery, drug development, regulatory affairs and manufacturing functions.
- Planning, implementation and completion of 6 new product programs for the US market including IND and NDA submissions
- Planning, implementation and completion of the first global drug development program within Ferring organization
- Preparation and filing of first international registration dossier within Ferring

Research and Development Responsibilities

- US Clinical Research and Development
- US Drug Development programs
- Pharmaceutical Development
- Regulatory Affairs
- Project Management
- Manufacturing Process Development

Vice President, Clinical and Regulatory Affairs

1993-1996

CIBUS Pharmaceutical, Inc., Redwood City, CA

Corporate Research Interests:

- Controlled delivery and tissue specific delivery of orally administered drugs
- Novel Therapies for Gastrointestinal Diseases

Research and Development Responsibilities

- Clinical Research and Development
- Regulatory Affairs
- Project Management

President

1992-93

PeptiMed Inc., Cambridge, Massachusetts.

Corporate Research Interests:

- Gastrointestinal Diseases
- Peptides of the Gastrointestinal Organs
- Systemic Diseases related to Proteins of the GI Tract

Corporate Responsibilities

- Develop broad-based GI disease research and development strategy
 - Established research programs supporting founding technology
 - Acquired additional early stage technology
 - Established new research program based on staff discoveries
- Recruit management and scientific staff
- Manage corporate research and development programs

- Develop business plan to raise equity capital from venture sources
- Business development
- Chairman, Corporate Research Committee
- Member, Scientific Advisory Board

Associate Research Fellow, Experimental Therapy, 1990-92
Warner-Lambert Pharmaceutical Research, Ann Arbor, Michigan
Director, Gastrointestinal Disorders Research

Research Interests and Research and Development Responsibilities:

- Gastrointestinal Diseases including mucosal repair and neoplasia
- Role of tissue non-specific and tissue specific growth factors in GI pathologies
- Quantitative image analysis in diagnostic imaging of the gastrointestinal, cardiovascular and central nervous systems
- Develop human research programs on pathophysiology and pharmacology
- Develop GI disease research strategy for WL/PD and coordinate GI-related research
 - Established 3 new research initiatives (2 progressed to clinical trials)
 - Identified 4 internal projects relevant to GI diseases (2 INDs resulted)
- Identify therapeutic targets
- Evaluate NCEs for their therapeutic potential and pharmacologic activity
- Collaborate with clinical research on the development NCEs

Senior Scientist, Section Head Clinical Biochemistry, 1986 - 90
Glaxo Inc., Research Triangle Park, North Carolina

Research Interests and Responsibilities:

- Gastrointestinal Diseases including mucosal repair and neoplasia
- Quantitative image analysis in diagnostic imaging of the gastrointestinal, cardiovascular and central nervous systems
- Develop human research programs on disease pathophysiology and pharmacology
- Coordinate activities of exploratory development project teams through the IND filing and early human trials (Project Leader for one program)
- Coordinated multidisciplinary research activities in discovery/development research programs (Project Leader for three programs)
- Evaluate NCEs for their therapeutic potential
- Collaborate with clinical research personnel on the development NCEs

Associate Director, Clinical Pharmacology, 1986
Glaxo Inc., Research Triangle Park, North Carolina

Associate Director, Clinical Investigations, 1983-86
Glaxo Inc., Research Triangle Park, North Carolina

Clinical Research and development Responsibilities:

- Evaluation of new compounds for clinical development and use in:
gastrointestinal diseases cardiovascular diseases

- hematologic disorders CNS and anesthesia
- Prepared sNDA for GERD indication for ZANTAC
 - prepared Gastrointestinal Diseases Advisory Committee presentation,
- Direct preparation of sNDA for Long-term Treatment of Duodenal Ulcers indication for ZANTAC
 - prepared Gastrointestinal Diseases Advisory Committee presentation,
- Provide technical support and advice to Marketing
- Identify and/or develop clinical pharmacology evaluation methods to facilitate drug development process
- Devise and implement clinical operating plan for evaluation of NCEs
- Prepare INDs for four NCEs

Assistant Director, Clinical Research and Development, 1980-83
Wyeth Laboratories, Inc., Radnor, Pennsylvania

Clinical Research and Development Responsibilities:

- Evaluation of NCEs for the treatment of cardiovascular diseases
- Review scientific literature on cardiovascular pathophysiology and pharmacology
- Provide technical advice and supportive documentation to the cardiovascular marketing group
- Devise and implement clinical operating plans for Phase I - III programs
- Assist in the preparation of the Guanabenz NDA
- Prepare Cardio-Renal Advisory Committee presentation for guanabenz
- Direct and review preparation of NDA for Guanabenz/HCTZ combination
- Prepared IND for two NCEs

Research Associate, 1979-83
Assistant Director of Hypertension Laboratories,
Division of Nephrology and Hypertension, Hahnemann Medical College and Hospital,
Philadelphia, Pennsylvania

Postdoctoral Fellow, 1977-79
Institute for Cancer Research, Fox Chase Cancer Center,
Philadelphia, Pennsylvania

Graduate Fellow, 1972-76
Rutgers University, New Brunswick, New Jersey
Dr. N. Ronald Morris - thesis advisor
Dr. Donald J. Wolff - interim advisor (sabbatical of Dr. Morris)
Thesis: Microtubule Assembly: Studies on the Mechanism of Griseofulvin Inhibition

Graduate Teaching Assistant,
CMDNJ-Rutgers Medical School, Rutgers University,
New Brunswick, New Jersey
Department of Bacteriology, 1972
Department of Pharmacology, 1973-75

AWARDS

United States Public Health Service Predoctoral Fellowship; 1972-74
Rutgers University, New Brunswick, New Jersey

National Cancer Institute Postdoctoral Traineeship, 1977-79
Institute for Cancer Research, Philadelphia, Pennsylvania

PROFESSIONAL ACTIVITIES

Member, Scientific Advisory Board,
Loats Associates Inc, Westminster, Maryland 1987 - 2000

Member, Executive Advisory Board, 1989 - 91
Member, Technical Advisory Board, 1989 - 91
Marmoset Research Colony at Oak Ridge, Oak Ridge, Tennessee

Member, Board of Directors 1992-93
PeptiMed Inc, Cambridge, Massachusetts

Chairman, Board of Directors 2001-02
Ferring Research Institute, San Diego CA

Chairman, Board of Directors 2001-02
Ferring Research LTD, Chilworth, England

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Stephen Brand and Ronald V. Nardi (1992). Treatment of Diabetes Mellitus. United States and foreign patents pending

INVITED PRESENTATIONS AND VIDEOTAPES

Quantitative Endoscopy: Morphometric Analysis of Gastrointestinal Lesions. Presented at the AGA/GRG Topic Forum on Applications of Newer Endoscopic Technology (1988).

Epidermal Growth Factor- The Future of Cytoprotection? Presented at the American College of Gastroenterology conference on Innovations in the Diagnosis and Treatment of Gastrointestinal Disorders (1989).

The Future of Video Endoscopy and Digital Imaging. Presented at the American College of Gastroenterology conference on Innovations in the Diagnosis and Treatment of Gastrointestinal Disorders (1989).

New Diagnostic Technologies in Endoscopy. Presented at the American College of Gastroenterology conference on Innovations in the Diagnosis and Treatment of Gastrointestinal Disorders (1989).

Quantitative Endoscopy: A New Approach (1989). Glaxo Research Laboratories Presented at Third International Congress on Video Endoscopy.

Using Early Drug Development Data to Design Phase III Studies (1990). Presented at Drug Information Association Workshop Statistical Issues in the Pharmaceutical Industry: Analysis and Reporting of Phase III Clinical Trials including Kinetic/Dynamic Analysis and Bayesian Analysis.

Strategic Integration of Drug Discovery and Development Activities (1993). Presented at Drug Information Association Annual Meeting.

Doctor, Lawyer, Merchant Chief: Who Should Direct the Licensing Organization and Licensing Strategy (1993). Presented at the Licensing Executive Society Annual Meeting.

Early Stage Company Perspective on the Value of CROs (1994). Presented at the Third Annual Conference on Re-engineering Drug Development Through Partnerships with CROs

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Ph.D. Thesis - Microtubule Assembly: Studies on the Mechanism of Griseofulvin Inhibition (1977).

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Exhibit B

FOCUS ON UROLOGICAL INDICATIONS

.....



Minirin[®]

DESMOPRESSIN

.....

DESMOPRESSIN

(MINIRIN[®], DDAVP[®])

FOCUS ON UROLOGICAL INDICATIONS

DESMOPRESSIN (MINIRIN[®], DDAVP[®])
FOCUS ON UROLOGICAL INDICATIONS

Sponsored as a service to medicine by Ferring AB

Published by Adis International Limited



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SUMMARY

.....

Several decades of experience with desmopressin (Minirin®, DDAVP®) have confirmed its efficacy and excellent tolerability profile. Desmopressin thus remains the drug of choice in the treatment of diabetes insipidus and primary nocturnal enuresis and is a convenient and reliable method of determining renal concentrating capacity. Furthermore, patients with urinary incontinence and/or nocturia may also benefit from treatment with desmopressin, but its usefulness in these indications requires further investigation.

- Desmopressin is a synthetic analogue of vasopressin and acts as a direct agonist at renal V_2 receptors, regulating the volume and osmolality of the urine.
- Desmopressin is devoid of the pressor effects of vasopressin.
- Desmopressin has a longer duration of action and a more potent antidiuretic activity than vasopressin.
- Desmopressin has been the treatment of choice in patients with central diabetes insipidus for more than 20 years, being well tolerated and highly effective in long-term use.
- Desmopressin supplements the low nocturnal levels of vasopressin present in many children with primary nocturnal enuresis, thus reducing the likelihood of enuresis.
- The desmopressin renal concentrating capacity test provides an early indication of renal dysfunction and is useful in the differential diagnosis of central/ nephrogenic diabetes insipidus.
- Desmopressin is preferred over the water deprivation and Pitressin® tests for the measurement of renal concentrating capacity.
- Abnormalities of endogenous vasopressin production may be central to urinary incontinence in some patients.

INTRODUCTION

.....

Desmopressin* (Minirin®, DDAVP®) is a synthetic analogue of the naturally occurring antidiuretic hormone vasopressin. All patients with central diabetes insipidus and many patients with primary nocturnal enuresis have a deficiency of endogenous vasopressin, and the efficacy of hormone replacement therapy with desmopressin in these patients is well established. Furthermore, its renal concentrating capacity has made it a useful and convenient tool for the early diagnosis of renal dysfunction. Patients with urinary incontinence and/or nocturia may also benefit from treatment with desmopressin, but its usefulness in these indications requires further investigation.

Desmopressin has proved to be safe and well tolerated in clinical practice. A potential risk with desmopressin is fluid retention, which can, however, be avoided by controlling fluid intake.

Desmopressin has provided a major advance in the treatment of conditions attributable to a deficiency of the endogenous hormone. This monograph provides readers with a complete and up-to-date analysis of studies with desmopressin in the treatment of diabetes insipidus and primary nocturnal enuresis and in tests of renal concentrating capacity. The use of desmopressin in the treatment of urinary incontinence and/or nocturia is also evaluated. Additionally, desmopressin is used in the treatment of various bleeding disorders, such as haemophilia A and von Willebrand's disease. This therapy, which requires 10–15 times higher doses than the antidiuretic treatment, has been extensively reviewed and will not be discussed further here.^[1,2]

* For the purposes of this monograph, all dosages of desmopressin refer to dosages of desmopressin acetate.

PHARMACOLOGY

Desmopressin (1-deamino-8-D-arginine-vasopressin) (Minirin®, DDAVP®) is a synthetic analogue of the naturally occurring antidiuretic hormone 8-arginine vasopressin. Vasopressin acts directly on the kidney to regulate the reabsorption and excretion of water.

Desmopressin (Minirin®, DDAVP®) has a longer duration of action than vasopressin and is without pressor effects

Vasopressin activity is exerted through V_1 and V_2 receptors. The former mediates effects on smooth muscle and the latter mediates antidiuretic activity (table 1). Desmopressin has no effect on V_1 receptors but has greater potency than vasopressin on renal V_2 receptors.[3]

Structurally, desmopressin differs from vasopressin in two principal ways: the absence of an amino group at position 1 and the substitution of D-arginine at position 8 (fig. 1). These modifications enhance the resistance of the molecule to enzymatic breakdown,

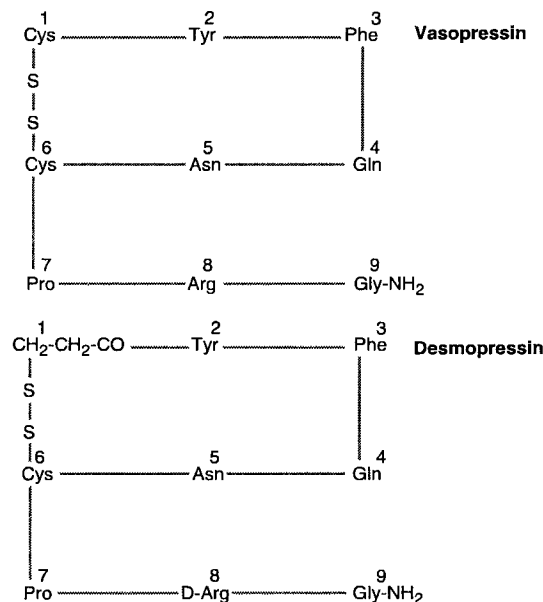


Fig. 1. Structural formulae of the naturally occurring hormone vasopressin and the synthetic analogue desmopressin.

increase the antidiuretic activity and eliminate the pressor effect.

Thus, desmopressin has a prolonged and more potent antidiuretic effect compared with

Table 1. Vasopressin receptor sites and activity

| Type of receptor | Tissue | Effect of stimulation |
|------------------|---|---|
| V_1 | Smooth muscle of blood vessels, uterus and intestine | Vasoconstriction; uterine contraction; increased intestinal peristalsis |
| V_2 | Kidney, thick ascending limb of Henle's loop and collecting tubules | Antidiuretic activity |

the natural hormone. Additionally, the pressor effect of vasopressin is avoided because desmopressin has no action on smooth muscle.

Role of vasopressin in the control of water reabsorption in the kidney

Vasopressin is synthesised in the hypothalamus and stored in the posterior pituitary gland.

Secretion is regulated by changes in plasma osmolality and changes in extracellular volume.^[4] Reductions in blood volume or blood pressure also stimulate vasopressin secretion. Such changes may occur during sleep: in healthy individuals there is a nocturnal increase in vasopressin that is associated with a decrease in urinary output.^[5]

Healthy individuals have an increase in nocturnal vasopressin secretion accompanied by a fall in urinary output

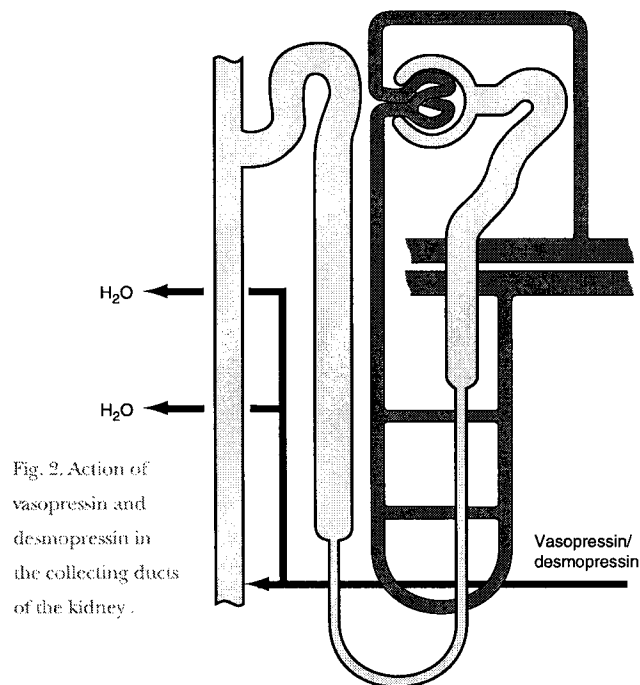


Fig. 2. Action of vasopressin and desmopressin in the collecting ducts of the kidney.

The collecting duct is the principal site of action of vasopressin (fig. 2). The presence of vasopressin, acting on V₂ receptors on the cells that make up the distal tubules and the collecting ducts, greatly increases the water permeability of these cells.

Flow of water from the distal tubules and collecting ducts also requires the presence of a surrounding hypertonic interstitium to create an osmotic driving force for water movement. After reaching the interstitium, water re-enters the systemic circulation via the peritubular capillary network.

P HARMACODYNAMICS

Preclinical studies

The antidiuretic activity of desmopressin (Minirin®, DDAVP®) has been investigated in numerous animal models, including hydrated rats and Brattleboro homozygous rats (a species that cannot synthesise vasopressin).

In Brattleboro rats, desmopressin (10 or 50 ng/kg) produced complete anuria for 2–4 hours; urine output returned to baseline after 10–14 hours.^[6] Furthermore, in order to achieve a similar antidiuretic effect in water-loaded rats, the dose of vasopressin needs to be 500 times greater than that of desmopressin.^[7]

Oral desmopressin (Minirin®, DDAVP®) significantly enhances antidiuretic activity while oral vasopressin results in only insignificant antidiuresis in rats

The antidiuretic potency of desmopressin after oral administration may be attributable in part to its resistance to degradation by digestive enzymes, as demonstrated by Matsui et al.^[8] In this study, the antidiuretic activity of vasopressin was completely abolished following incubation with digestive enzymes. In contrast, the activity of desmopressin was either unaffected or only partially affected.

Clinical studies

Antidiuretic effects

The antidiuretic effect of desmopressin has been demonstrated in studies in hydrated,

healthy volunteers. In a representative study, mean urinary output was reduced from 15 ml/min to 2 ml/min 30 minutes after the administration of intranasal desmopressin 20 µg. A minimal rate of urine production of around 0.6 ml/min was seen 5 hours after treatment, while urine osmolality increased from a baseline value of 100 mOsm/kg to around 800 mOsm/kg.^[9]

In a study in hydrated volunteers receiving oral desmopressin (20, 40 and 200 µg) there was a dose-dependent reduction in urine volume and a concomitant increase in urine osmolality.^[10]

Hormonal and cardiovascular responses

The hormonal, biochemical and cardiovascular responses to desmopressin have been investigated in both volunteers and patients with primary nocturnal enuresis.

Fluid-deprived adult volunteers (n=6) were treated with intravenous desmopressin 0.4 µg/kg, a dose 10-fold higher than that required for antidiuretic purposes, to determine hormonal and cardiovascular responses. Five minutes after the infusion, subjects exhibited facial flushing, a 13% decrease in mean diastolic blood pressure and an 18% increase in mean pulse rate. A significant increase in plasma renin activity and plasma cortisol levels was observed, but there were no significant changes in plasma levels of luteinising hormone, follicle-stimulating hormone, thyroid-stimulating hormone, prolactin or growth hormone.^[11]

Similar haemodynamic effects were seen in the same study when intravenous desmopressin was administered to 5 patients with central diabetes insipidus.

**Desmopressin (Minirin®, DDAVP®)
does not affect endogenous
vasopressin secretion. Routine
laboratory tests are normal after
desmopressin administration**

The hormonal and biochemical effects of vasopressin were further investigated in 7 patients with primary nocturnal enuresis who were treated with intranasal desmopressin 10 µg or 20 µg for 4–24 (mean 13) months. Desmopressin had no significant effect on endogenous vasopressin secretion, and routine laboratory tests were normal in all patients. The cortisol estimations showed a normal diurnal variation in each case.^[12]

P HARMACOKINETICS

The pharmacokinetics of desmopressin (Minirin®, DDAVP®) have been investigated after oral, intranasal, intravenous and subcutaneous administration to healthy volunteers (table 2) and after oral and intranasal administration to patients with diabetes insipidus.^[13,14]

Absorption

Significant plasma concentrations of desmopressin are observed after oral administration to healthy adults. Desmopressin is detectable in plasma within 30 minutes of either intranasal or oral administration; maximal concentration and maximal response are dose-dependent and are achieved within 2 hours. Lam and colleagues investigated the pharmacokinetics of intranasal and oral desmopressin in 10 Chinese adults with

Maximum plasma desmopressin (Minirin®, DDAVP®) concentrations are achieved within 2 hours of intranasal or oral administration

central diabetes insipidus.^[14] Following 20 µg intranasally and 200 µg orally, respective plasma desmopressin concentrations (mean ± standard error) peaked after 45.6 ± 7.3 and 93.3 ± 3.3 minutes, reaching concentrations of 24.1 ± 4.7 and 15.1 ± 3.2 pmol/L. Respective terminal half-lives were 2.2 ± 0.1 and 2.0 ± 0.1 hours. Based on the area under the concentration–time curve, the bioequivalent intranasal:oral ratio was 1:16.

Absorption of desmopressin after oral administration occurs primarily in the

Table 2. Mean pharmacokinetic parameters of desmopressin in 8 healthy volunteers following different routes of administration (reproduced with permission)^[13]

| Route | Dose (µg) | Pharmacokinetic parameter | | | |
|--------------|-----------|--|---------------------------|------------------------|------------------------------|
| | | AUC (pmol·L ⁻¹ ·h ⁻¹) | C _{max} (pmol/L) | T _{max} (min) | Bioavailability ^a |
| Intravenous | 2 | 114.4 | – | – | – |
| Subcutaneous | 2 | 189.4 | 58.3 | 41.4 | NR |
| Intranasal | 20 | 58.9 | 19.9 | 60.0 | 3.4% |
| Oral | 200 | 23.8 | 12.7 | 71.4 | 0.1% |

^a Compared with the intravenous route.

Abbreviations: AUC = area under the plasma–concentration time curve; C_{max} = peak plasma concentration; NR = not reported; T_{max} = time to C_{max}.

Desmopressin (Minirin®, DDAVP®) bioavailability may be optimised by administration half an hour before or 2 hours after a meal

duodenum and the proximal jejunum. Consequently, absorption may be reduced in conditions of rapid intestinal transport. Absorption is reduced if desmopressin is administered with food.^[15] Therefore, in cases where the effect of desmopressin is less

Desmopressin (Minirin®, DDAVP®) undergoes biphasic elimination

than optimal, the bioavailability of orally administered desmopressin may be improved by appropriate timing of the dose (i.e. at

least half an hour before or 2 hours after a meal).

Distribution

The apparent volume of distribution of desmopressin is relatively small (0.2 L), indicating that it does not enter the intracellular compartment.^[16] Furthermore, results obtained in patients with communicable hydrocephalus indicate that desmopressin does not penetrate the blood–brain barrier.^[17]

Clearance

The elimination of desmopressin is bi-exponential, with a rapid first phase and a slower second phase, with half-life values of 8 minutes and 1–2 hours, respectively.^[13,18] Urinary clearance is variable (1.19–3.83 ml·min⁻¹·kg⁻¹) after intravenous, intranasal or oral administration of desmopressin.^[13]

UROLOGICAL INDICATIONS

The three main indications for desmopressin (Minirin®, DDAVP®) as an antidiuretic are:

- central (cranial) diabetes insipidus
- primary nocturnal enuresis
- renal concentrating capacity test (RCCT).

Desmopressin has also been investigated for the treatment of urinary incontinence and/or nocturia. It is approved in the UK for nocturia associated with multiple sclerosis.

Central diabetes insipidus

Central diabetes insipidus (also known as cranial or neurogenic diabetes insipidus) arises from a deficiency in vasopressin secretion.

Symptoms include thirst, polydipsia and polyuria with nocturia. The daily water turnover is 4–20 L, depending on the severity of the vasopressin defect. A dangerous hyperosmolality or dehydration may develop within hours and consciousness may be lost. Patients with central diabetes insipidus respond to the administration of desmopressin with a prompt increase in urine osmolality. However, patients with renal (nephrogenic) diabetes insipidus show little or no response to desmopressin administration.

Clinical experience with desmopressin

Previous therapies for central diabetes insipidus (posterior pituitary extracts, lysine vasopressin and non-hormonal drugs) were limited by a short duration of action, adverse effects and poor antidiuretic efficacy. The first

clinical study of desmopressin indicated a considerable improvement over vasopressin therapy in patients with diabetes insipidus.^[6] Subsequent clinical trials in this indication showed desmopressin to be superior to vasopressin and other non-hormonal agents and established desmopressin as the drug of choice in central diabetes insipidus.^[19,20]

Desmopressin (Minirin®, DDAVP®) is superior to vasopressin and non-hormonal agents for the treatment of central diabetes insipidus

Intranasal administration

The intranasal dose required to control diabetes insipidus varies considerably between patients and does not appear to correlate with age, bodyweight, body surface area or the severity of polyuria.^[21] The effect of intranasal desmopressin in patients with central diabetes insipidus was reported by Robinson.^[22] Desmopressin was effective in all cases, the effect being noted within the first hour of administration and persisting for 8–20 hours. The effect of the drug usually ceased rather abruptly over 60–90 minutes, when an increased flow of urine was noted by the patient. The response pattern varied from case to case, though repeat administrations to a given patient yielded similar results. The crucial determinant of the frequency of administration (1, 2 or 3 times daily) necessary to control

Individualisation of the dosage regimen is required to optimise the therapeutic response to desmopressin (Minirin®, DDAVP®) in central diabetes insipidus

urine output was the duration of the response. No side effects, such as increased pulse rate, increased blood pressure, abdominal cramps or flushing, were noted by any patient during the initial administration of desmopressin or during 6 months' maintenance treatment, and no patient manifested changes in haemoglobin, red cell count, white cell count

or serum levels of sodium, potassium, chloride and carbon dioxide, plasma osmolality, blood urea nitrogen, creatinine, albumin, cholesterol, bilirubin, aspartate aminotransferase (GOT), alkaline phosphatase or fasting blood sugar.

In 36 children with central diabetes insipidus, intranasal desmopressin was superior to any prior treatment with respect to urine volume reduction and urine concentration maintenance in the same patients (table 3). Furthermore, the ease of administration increased patient compliance with desmopressin therapy.^[21]

Clinical experience has shown that the average daily intranasal dosage for central

Table 3. Urine volume and osmolality in 36 children with central diabetes insipidus receiving a variety of treatments (reproduced with permission of S Karger AG, Basel)^[21]

| Therapy (route of administration) | No. of patients | Daily dose | Urine volume (L/24 hours) | Urinary specific gravity | Urine osmolality (mOsm/kg H ₂ O) |
|-----------------------------------|-----------------|------------|---------------------------|--------------------------|---|
| Baseline | 36 | | 4.0–12 | 1000–1003 | 44–220 |
| Pitressin® tannate in oil (IM) | 18 | 2–5 IU | 2.0–3.5 | 1007–1018 | 215–390 |
| Pitressin® powder (IN) | 14 | 40–80 mg | 1.8–3.6 | 1004–1018 | 190–390 |
| Lysine-8-vasopressin (IN) | 3 | 8–20 IU | 2.4–3.8 | 1008–1016 | 140–289 |
| Chlorpropamide (oral) | 2 | 200–400 mg | 4.0–6.0 | 1006–1008 | 156–203 |
| Desmopressin (IN) | 34 | 2.5–30 µg | 0.9–1.7 | 1012–1025 | 420–1005 |

Abbreviations: IM = intramuscularly; IN = intranasally.

diabetes insipidus is 10–20 µg once or twice daily in adults and 5–10 µg once or twice daily in children.

Oral administration

Clinical studies have clearly demonstrated the efficacy of oral desmopressin in the treatment of diabetes insipidus.^[23–25] Adverse reactions were few and similar to those reported with intranasal treatment.

As was the case with intranasal administration, oral dosage requirements were unrelated to age, severity of polyuria or bodyweight. Although it was not possible to establish a relationship between the efficacy of intranasal and oral administration that could be used to predict individual dosage requirements, it was suggested that the oral dosage needed to treat diabetes insipidus would be larger than the intranasal dosage.^[25]

A 10-year follow-up was performed on 6 patients who were included in a study published in 1986.^[24] The patients (now 14–27 years of age) were treated with oral desmopressin throughout this period. The total 24-hour dose was 0.2–1.6 mg, and the frequency of administration was 3 times daily in all but one case (for whom it was twice daily). All patients were well controlled, with the volume of urine ranging from 620 to 1500 ml/24 hours. The patients were also very satisfied with the efficacy and convenience of oral desmopressin therapy. A series of

clinical chemistry tests showed no significant changes that could be related to desmopressin treatment, and no adverse events were reported.^[9]

As with intranasal therapy, the oral dosage required to control diuresis is highly individual. For an adult patient with diabetes insipidus, a suitable dosage is 0.1–0.2 mg two to three times daily. In rare instances, a higher dosage (0.8–1.6 mg/day) might be necessary. Patients who use intranasal desmopressin can be switched to the oral treatment overnight.

It is possible to switch patients from intranasal to oral administration of desmopressin (Minirin®, DDAVP®) overnight

Fjellestad-Paulsen and colleagues assessed the safety and efficacy of long-term treatment with oral desmopressin in eight patients (aged 3–21 years) with central diabetes insipidus.^[26] Five normal children of both sexes (aged 4–19 years) served as controls. As expected, urine osmolality was lower and urine volumes were larger among the patients vs the controls. No differences were seen between patients and controls with respect to plasma osmolality and sodium levels. The mean concentrations of atrial natriuretic peptide and aldosterone in the plasma were somewhat lower in the patients than in the controls, although the difference was not statistically significant. In

addition, there was no significant difference in plasma renin activity between the two groups.

The efficacy of the desmopressin tablet was very similar after 1 year and after 3.5 years of treatment. The disease was well controlled in all cases, mean daily diuresis being 1.7 L, with an absence of nocturnal polyuria. There was no relationship between the oral dose required and the previous intranasal dose, or the age or weight of the patient. No adverse reactions or clinically important deviations in laboratory values were reported. No circulating antibodies to desmopressin were detectable. It was concluded that long-term treatment with oral desmopressin is safe and effective.

Lam and co-workers performed a 1-year prospective study in 10 Chinese adults with central diabetes insipidus previously controlled with intranasal desmopressin.^[14] Oral desmopressin (300–600 µg/day in 2–3 doses) produced and maintained a stable and satisfactory antidiuresis, comparable to that seen with the previous intranasal therapy. The oral treatment was well tolerated, with no events warranting drug withdrawal.

Dose equivalence between intranasal and oral treatments

The use of oral desmopressin was investigated in 12 patients with diabetes insipidus who were previously well controlled with intranasal therapy.^[27] The oral dose of desmopressin was increased until the daily urinary output volumes became equal to those produced

during intranasal therapy. The antidiuretic dose-equivalence ratio for intranasal:oral desmopressin ranged between 1:15 and 1:30 (mean ratio 1:18). This ratio is in agreement with results obtained in enuretic children by Janknegt and colleagues, who found the efficacy of a 20 µg intranasal dose to be similar to that of an oral 400 µg dose.^[28]

Parenteral desmopressin

Desmopressin is administered parenterally in the initial treatment of early postneurosurgical central diabetes insipidus before intranasal administration is initiated. Parenteral desmopressin is initiated at relatively low doses (0.1–0.5 µg) and the antidiuretic effect usually lasts for 8–12 hours. In a study by Chanson et al., postoperative central diabetes insipidus was corrected 6 hours after initiation of a 3-day course of desmopressin 1, 2 or 4 µg intramuscularly every 12 hours.^[29] The effect on diuresis and osmolality was maximal from 18 hours onwards. Tolerability was excellent: 11/15 patients (73%) had mild hyponatraemia without clinical sequelae.

Nocturnal enuresis

Monosymptomatic nocturnal enuresis is defined as exclusive night-time wetting in the absence of the following factors:

- daytime incontinence of any type or severity
- increased frequency of micturition (voiding ≥ 8 times a day) plus urgency (a sudden desire

to void that has to be obeyed immediately in order to avoid incontinence)

- voiding postponement, with infrequent voidings (≤ 3 voidings per day)
- habitual holding manoeuvres such as sitting on the heel (i.e. squatting) or pinching the penis
- prolonged initiation of voiding and/or straining and/or interrupted (fractionated) voiding.

A lower age limit for the definition has not yet been set, but the usual age at which this is considered a clinical problem is around 5 years.

Monosymptomatic nocturnal enuresis has been categorised into two types:

- primary nocturnal enuresis – a disorder in children who have never been consistently dry
- secondary or onset enuresis – a disorder in those who start wetting the bed again after a significant dry period.

Monosymptomatic nocturnal enuresis is common; 20% of boys and 10% of girls are enuretic at 6 years of age. Primary nocturnal enuresis accounts for around 90% of these cases,^[30] and incidence declines with age, but 2–3% of patients continue to wet the bed during their late teens and early adulthood.^[31] These 2–3% will probably have a lifelong problem (fig. 3).^[32]

Spontaneous resolution of nocturnal enuresis has been reported in up to 14–16% of cases annually.^[30,31]

Nocturnal enuresis has been described as one of the most common of all childhood problems

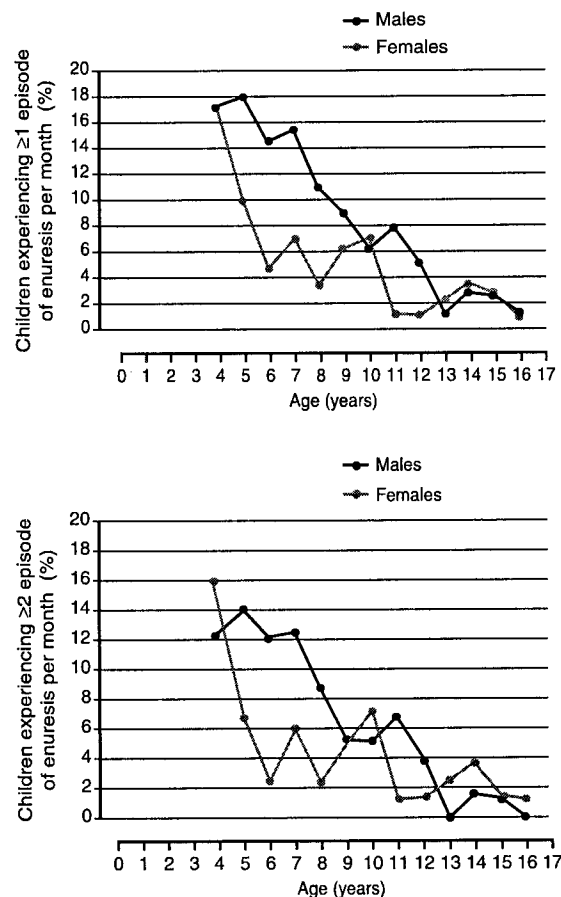


Fig. 3. Prevalence of nocturnal enuresis in a random sample of 2070 children according to age and gender.^[32]

The impact of nocturnal enuresis

Nocturnal enuresis often leads to considerable emotional disturbance,^[30,33–36] has a deleterious impact on self-esteem,^[37] and puts a significant financial burden on the child's family.^[38,39]

Nocturnal enuresis results in significant emotional disturbances and puts a significant financial burden on the child's family

Pathogenesis

The pathogenesis of nocturnal enuresis has recently been extensively reviewed by Nørgaard et al.^[40]

Currently, there is a consensus among opinion leaders from all specialities worldwide that psychopathology is not a major factor in the aetiology of nocturnal enuresis. Indeed, it has been noted that the mental health of bedwetting children improves after their enuresis has been treated successfully.^[37,41]

From a theoretical viewpoint, there appear to be four important factors influencing the pathogenesis of nocturnal enuresis:

- sleep pattern
- bladder behaviour and characteristics
- nocturnal diuresis
- genetic disposition.

Sleep pattern

Contrary to previous views, children with nocturnal enuresis have the same sleeping patterns as non-enuretic children, and enuretic episodes are equally distributed over all stages of sleep.^[42,43] However, results of a recent questionnaire-based survey of 7- to 10-year-olds indicate a significant difference in subjective arousability between enuretic and non-enuretic children, with the former group characterising themselves as very difficult or almost impossible to arouse from sleep and the latter group usually considering themselves easy or fairly easy to awaken.^[44]

Children with nocturnal enuresis have the same sleeping patterns as non-enuretic children

Bladder behaviour and characteristics

Urodynamic studies in enuretic patients have been performed with both invasive and non-invasive techniques, but these have not given any positive findings: bladder size is found to be normal even in large populations of enuretics.^[45,46] Additionally, daytime bladder

Urodynamic investigations have indicated that the cause of bedwetting in the majority of cases is not related to bladder dysfunction

function is normal in these individuals and no correlation has been found between nocturnal instability and the time of enuresis.^[43]

Nocturnal diuresis

It has been known for many years that a substantial number of enuretic patients produce large amounts of urine during sleep. However, little attention was paid to this finding until the last 10 years, when new theories emerged concerning important factors in the pathogenesis of nocturnal enuresis.

The normal regulation of urine production leads to a significantly decreased urinary output during sleep, and the concentration of urine also becomes optimal.^[47] This finding could not be reproduced in studies of patients with nocturnal enuresis, and consequently water metabolism in children became a subject for further investigation.

Nocturnal secretion of vasopressin

Normally, humans have a diurnal rhythm in the rate of urinary output that is reciprocal to urine osmolality; the secretion of endogenous vasopressin is increased at night, resulting in reduced urinary output and increased urine osmolality (fig. 4).^[43,48] Patients with nocturnal enuresis have a less pronounced increase in nocturnal endogenous vasopressin or even a reversal in the diurnal rhythm. This explains the large volumes of dilute urine that are produced, which in turn lead to overfilling of the bladder and hence enuresis.^[43,49]

It is important to note that bladder capacity is often normal in patients with nocturnal enuresis, which emphasises the role of vasopressin and nocturnal polyuria in the underlying aetiology.^[48,50,51]

Genetic disposition

Most evidence supports a biological aetiology and genetic predisposition for nocturnal enuresis (table 4).^[52] Males are affected more than females and the risk of developing nocturnal enuresis increases if one or both parents were enuretic as children (45–75%, respectively).^[52,53] A genetic locus corresponding to nocturnal enuresis has variously been identified on chromosome 12q^[54] and chromosome 13q,^[53] substantiating an inherited dysfunction.

Treatment

When the child gains sufficient maturity and motivation to co-operate (usually around 7 years of age), a variety of treatments can be offered.^[36] Understanding, optimism and reassurance are considered necessary elements in the initial approach to treatment of any enuretic patient. There are two main modes for the active treatment of bedwetting:

- conditioning devices (e.g. enuresis alarms)
- medical treatment.

Patient counselling and reassurance form an integral part of the treatment of nocturnal enuresis

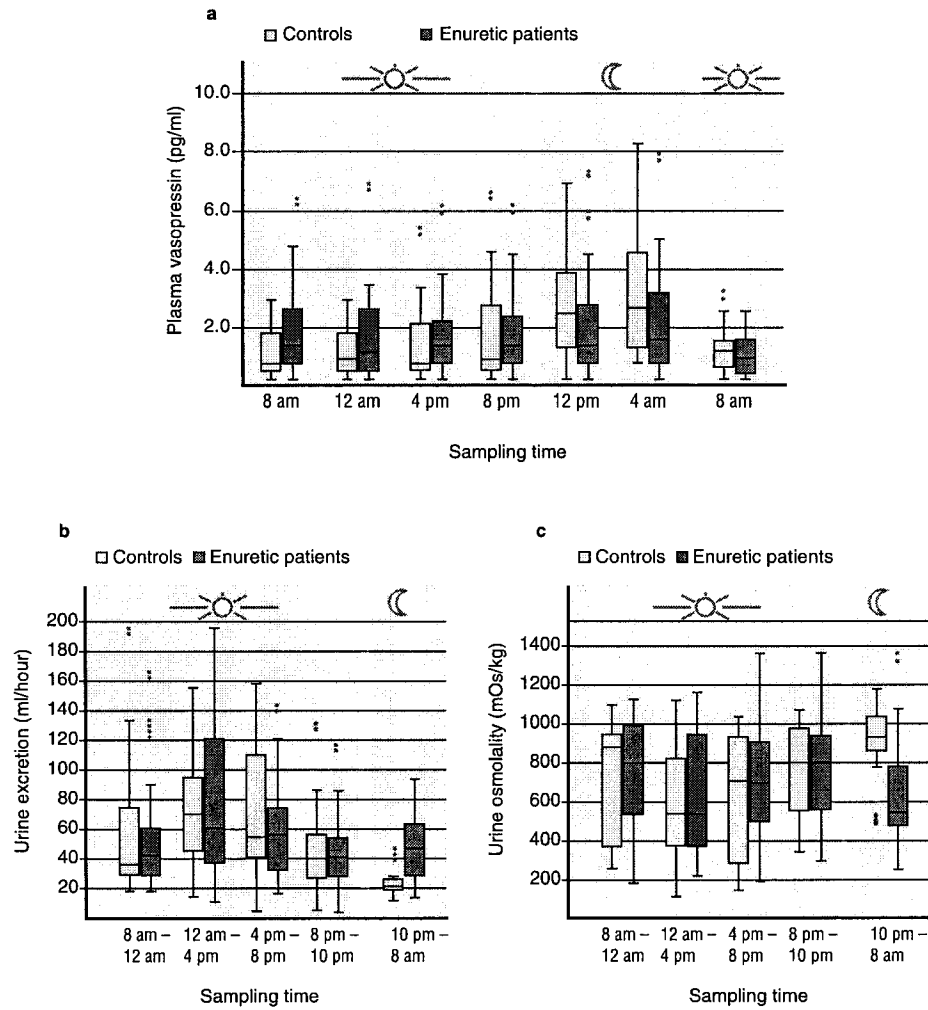


Fig. 4. Diurnal levels (total range and median value and first quartile) of plasma vasopressin (a), urine excretion rate (b) and urine osmolality (c) in 15 patients with nocturnal enuresis and 11 healthy volunteers. Dots represent extreme values exceeding the interquartile length by 1.5 times (reproduced with permission).^[43]

Table 4. Support for a biological aetiology in primary nocturnal enuresis

Hereditary

- A clear genetic predisposition for nocturnal enuresis exists^[52]

Sleeping pattern

- Children with primary nocturnal enuresis have the same sleeping pattern as normal children^[42,43]

Urodynamics

- Bladder instability is not a significant factor in the pathogenesis of primary nocturnal enuresis^[43]
- Small functional bladder capacity was not a requirement for the attainment of dryness in children treated with the enuresis alarm^[50]

Conditioning treatment

Conditioning treatment with enuresis alarms is considered by many to be the first treatment choice in enuretic children. However, the initial arrest of enuresis by the alarm does not result in a permanent cure in all patients. For example, among children treated with the alarm, only 55% remained dry after 6–12 months.^[50,55] Moreover, 44% of children cured by enuresis alarms had a negative opinion of this method.^[56]

Heavy demands are placed on parents by alarm treatment and its use may be limited by difficulties in obtaining adequate family co-operation.^[57] When these factors are taken into consideration and the family feels able to co-operate, the treatment is often successful and in many patients can result in long-term dryness. However, long-term studies are

needed to verify the exact cure rate of this treatment modality.

Medical treatment can be offered to patients unresponsive to alarm treatment or who come from families unable to support alarm treatment.

Medical treatment

Three types of pharmacological agents have been evaluated in studies of bedwetting:

- parasympatholytics
- tricyclic antidepressants
- the antidiuretic desmopressin (Minirin®, DDAVP®).

Parasympatholytics

Oxybutynin is an antimuscarinic agent that diminishes the frequency of involuntary bladder muscle contractions and increases bladder capacity.^[58] However, it is associated with antimuscarinic adverse events (e.g. dry mouth, visual impairment, constipation) that often prevent optimisation of the dosage regimen. Only one study has evaluated the efficacy of oxybutynin in paediatric nocturnal enuresis.^[59] This study, which was placebo-controlled, found no significant differences

Current evidence suggests that parasympatholytics as single therapy are not effective in nocturnal enuresis

between oral oxybutynin 10 mg and placebo in terms of the frequency of nocturnal enuresis.

Tricyclic antidepressants

Tricyclic antidepressants have some effect on enuresis, probably arising from autonomic modulation. However, they are unsuitable for the treatment of nocturnal enuresis because of their association with serious adverse effects, including psychomotor and cognitive impairment, sedation and cardiac toxicity. Tricyclic antidepressants are therefore not recommended for the treatment of patients with nocturnal enuresis.

Tricyclic antidepressants are not recommended in primary nocturnal enuresis

Desmopressin

Desmopressin is effective and well tolerated in children with nocturnal enuresis.^[12,60] The long-term efficacy and tolerability of desmopressin in the treatment of severe nocturnal enuresis have also been proved favourable.^[61]

Studies investigating the efficacy of oral or intranasal desmopressin generally comprise an initial dosage-titration phase (around 4 weeks) to determine the optimum dosage for a given individual. The patient subsequently continues to receive this dosage during the maintenance phase of the study. A review by Houts et al. investigated the efficacy of desmopressin in

14 studies of patients with nocturnal enuresis.^[62] In these studies, the number of enuretic episodes was reduced by up to 3.2 per week. Selected studies are summarised in table 5.

Desmopressin (Minirin®, DDAVP®) has shown favourable results in terms of efficacy and tolerability in children with nocturnal enuresis

Desmopressin is available in both intranasal and oral dosage forms for the treatment of nocturnal enuresis. Both are effective and well-tolerated in clinical use.^[61,64]

Intranasal desmopressin The results of several studies indicate that intranasal desmopressin is the drug of choice in nocturnal enuresis.^[36] Indeed, one randomised, double-blind, crossover study of 28 children and adults with nocturnal enuresis reported that the titrated effective dosage of intranasal desmopressin significantly decreased the number of wet nights per week by >90% in 68% of the patients.^[69]

In another study by Terho, 52 children aged 5–13 years (most of whom were refractory to previous treatment) were randomised to four periods of 3 weeks each: two periods on placebo and two periods on intranasal desmopressin 20 µg.^[67] There was a significant

Table 5. Summary of studies of desmopressin in children with primary nocturnal enuresis

| Reference | No. of patients | Dosage regimen ^a | Treatment duration (weeks) | Reduction in mean number of wet nights/week (%) | Patients responding (%) | |
|---|-----------------|-----------------------------------|----------------------------|---|-----------------------------|--|
| | | | | | Full response ^b | Partial response ^c |
| Fjellestad-Paulsen et al. ^[25] | 30 | 200 µg/day (PO) or 20 µg/day | 2 | | 31 | 62 (1–2 wet nights/week or an increase in dry nights by 1–2 nights/week) |
| Hjältnäs et al. ^[63] | 393 | 20 or 40 µg/day | ≤52 | | 19 | |
| Matthiesen et al. ^[64] | 18 | 200 or 400 µg/day (PO) | 6 | 67 | 0 | 100 (considerable improvement) ^d |
| Miller & Klauber ^[65] | 176 | 20 µg/day 40 µg/day Placebo | 4 | 21–29 34–41 13–15 | | |
| Miller et al. ^[66] | 55 | 40 µg/day | ≤52 | | 51 | |
| Terho ^[67] | 52 | 20 µg/day | 3 | 58–63 | 29 | 38 |
| | 47 ^e | 20, 30 or 40 µg/day | 12 | | 53 (≥5 dry nights/week) | 19 |
| Tuverno ^[68] | 18 | 20 µg/day | 4 | | 44 (27 or 28 dry nights/28) | 44 (16–26 dry nights/28) |

^a Desmopressin was administered intranasally, except where indicated.

^b Complete dryness unless otherwise indicated.

^c >50% reduction in wet nights unless otherwise indicated.

^d Mean frequency of wet nights reduced from 5.3 nights/week at baseline to 1.7 nights/week during desmopressin therapy.

^e Patients who relapsed after cessation of desmopressin therapy.

Abbreviation: PO = orally.

increase ($p<0.01$) in the number of dry nights per week from 0.6 at baseline to 4.3 and 4.6 during the two 3-week periods of treatment with desmopressin. During the corresponding 3-week placebo periods, 2.1 and 2.4 dry nights per week were recorded.^[67]

Miller et al. evaluated the long-term efficacy of desmopressin in 55 children who had

Intranasal desmopressin (Minirin®, DDAVP®) has proved effective in patients refractory to previous treatments

initially received intranasal desmopressin 40 µg per night for 2 weeks.^[66] Responders continued to receive intranasal desmopressin for up to 12 months; the dosage was gradually reduced by 10 µg every 2 weeks once total dryness was achieved. In total, 28 children (51%) had a positive response to initial therapy and then progressed to total dryness with long-term therapy, while 8 children (14%) had an initial positive response but did not progress to total dryness on long-term follow-up. Complete weaning from desmopressin required >6 months in most patients, and a minimum of 3 months.^[66]

Similarly, the Swedish Enuresis Trial (SWEET) assessed the long-term efficacy and tolerability of intranasal desmopressin in 393 children aged 6 to 12 years with primary nocturnal enuresis.^[63] After a 6-week dose-titration phase, during which patients received

desmopressin 20 or 40 µg at bedtime, those achieving a ≥50% reduction in the frequency of wet nights ($n=242$) continued open-label treatment (typically with the 40 µg dose) for up to 12 months. During dose-titration, the median weekly number of wet nights fell from 4.8 to 1.0. On completion of the trial, 133 of the 393 children (34%) had achieved a ≥90% reduction in the number of wet nights, and 75 children (19%) were completely dry at night. The majority of children who became dry reached this stage during the first 6 months of treatment.

Oral desmopressin It is recommended that oral desmopressin is initiated at a dosage of 200 µg/day, increasing to 400 µg/day if necessary. The antidiuretic efficacy of oral desmopressin 200 or 400 µg/day is similar to that attained with the intranasal formulation and provides a useful alternative to the intranasal route of administration.

A dose-response relationship was observed in a single-blind study of oral desmopressin administered at dosages of 50–400 µg/day in 15 children with nocturnal enuresis. The 200 µg dosage resulted in a significantly greater ($p<0.02$) number of dry nights than with 100 µg/day, but did not differ significantly from the 400 µg/day oral dosage.^[25] However,

Oral desmopressin (Minirin®, DDAVP®) therapy should be initiated at 200 µg/day and increased to 400 µg/day if necessary

another single-blind, dose-ranging study found that a daily oral dosage of desmopressin 400 µg was generally somewhat more effective than a 200 µg dose in adolescents with severe nocturnal enuresis.^[61] A subsequent 4-week, double-blind, crossover study in these patients (n=10) reported that oral desmopressin, at a dosage of 200 or 400 µg/day, produced a greater reduction in the number of wet nights (from 4.7 to 1.8 per week) than did placebo (from 4.7 to 4.1 per week).^[61]

A 6-week study by Matthiesen et al. found oral desmopressin 200 or 400 µg/day to be at least as effective as the maximum effective dosage of the nasal spray in around 50% of enuretic patients.^[64] In general, oral desmopressin 400 µg was more effective than the 200 µg dosage. The mean number of wet nights was reduced from 5.0 at baseline to 1.8 during oral treatment with desmopressin 200 or 400 µg/day. Three of the 18 patients who were non-responders to oral desmopressin achieved a full response with the nasal spray. There were no adverse events and no tendency towards hyponatraemia with either formulation.^[64]

General comments on the treatment of nocturnal enuresis

Today, more is known about nocturnal enuresis and research has established various patient subtypes. First, enuretics can be divided into monosymptomatic bedwetters and those who also have daytime incontinence. The latter group

requires a treatment directed towards their bladder dysfunction, whereas the former group will probably benefit from desmopressin treatment. Through an increased understanding of voiding dysfunction in children, a differentiated and more optimal treatment of bedwetting can be offered. Combined studies on bladder function and sleep in children suffering from bedwetting have revealed that their bladder function is normal and that enuretics have a similar sleep pattern to non-enuretics.^[70]

Urinary incontinence and nocturia

Urinary incontinence is a distressing and embarrassing condition that is common among the general population. There is a general increase in the prevalence of urinary incontinence with increasing age, and around 10% of the elderly population living at home are thought to be affected.^[71]

Although the actual prevalence of urinary incontinence is relatively low in the elderly, up to 70% of this group have nocturia and 40% micturate more than twice nightly.^[71] The need to rise in the night to micturate leads to sleep disturbances that can affect performance the following day.^[72]

Pathogenesis

Urinary incontinence is generally attributable to disorders of bladder storage. One of the most common causes of involuntary leakage of urine is uncontrolled spontaneous

contractions of the bladder during filling, which is known as 'detrusor instability'. In addition to urinary incontinence, detrusor overactivity gives rise to symptoms of urgency, increased frequency of micturition and nocturia. These symptoms may have precipitating factors linked to the function of the cardiovascular, central nervous, endocrine and metabolic systems.

Patients without the need for nocturnal micturition have a daytime urinary output

Patients with nocturia frequently have undetectable vasopressin levels

twice as high as that at night.^[73] However, in older patients with nocturia, the day:night urinary output ratio is reduced, and in such patients there is an increased frequency of nocturnal micturition. Plasma vasopressin is at undetectable levels in many such patients.^[72]

From the above, it appears that abnormalities of vasopressin production may be central to urinary incontinence in some patients; desmopressin is therefore a rational therapeutic choice in patients with urinary incontinence and/or nocturia. By decreasing urinary output, desmopressin therefore allows patients to have a predictable, reliable period of hours free from nocturia and/or urinary incontinence.

Clinical studies

Studies have demonstrated the favourable efficacy and tolerability of desmopressin in the treatment of urinary incontinence and/or nocturia arising in a number of patient groups. In particular, the successful treatment of urinary incontinence and/or nocturia with desmopressin in patients with multiple sclerosis (MS), 80% of whom have neurogenic bladder dysfunction, has been reported.^[74-77]

Intranasal desmopressin

Placebo-controlled, double-blind studies support the general efficacy and tolerability of intranasal desmopressin in nocturia^[78] and urinary incontinence.^[79,80] Studies in specific patient groups have demonstrated the efficacy of desmopressin administration in urinary incontinence arising as a consequence of MS (as outlined above), prostatic hyperplasia^[81] and Alzheimer's disease.^[82]

A double-blind, crossover study of 33 patients with MS compared intranasal desmopressin (20 µg on retiring to bed) with placebo for the treatment of nocturia. Placebo treatment produced little change in frequency of micturition from baseline; in contrast, desmopressin produced significant reductions

Desmopressin (Minirin®, DDAVP®) therapy is effective in the treatment of nocturia in patients with multiple sclerosis

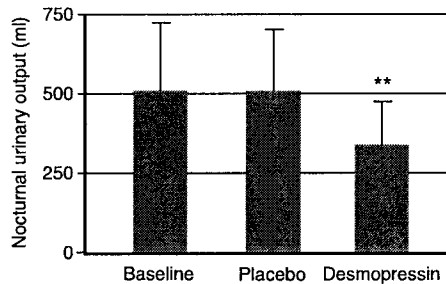


Fig 5. Effects of placebo and desmopressin 20 µg on nocturnal urinary output in a double-blind crossover study of 33 patients with nocturia as a consequence of multiple sclerosis: ** $p < 0.01$ vs placebo.^[75]

($p < 0.01$) in mean nocturnal frequency of micturition and voided volume compared with placebo (fig. 5), with little change in the daytime frequency of micturition or voided volume.^[75]

Hilton and Stanton reported that desmopressin was effective in patients refractory to antispasmodic and evening fluid-

restriction interventions.^[80] This 6-week, double-blind, crossover study compared desmopressin to placebo in 25 female patients with nocturia. Compared with both placebo and baseline measurements, nocturnal urinary output and frequency of micturition were both reduced during desmopressin therapy, with a slight increase in diurnal urinary output and little change in overall diurnal urinary frequency (table 6).

Another double-blind study assessed desmopressin vs placebo in 20 men with increased nocturnal frequency of micturition.

Desmopressin (Minirin®, DDAVP®) is effective in patients with urinary incontinence who are refractory to antispasmodic therapy and evening fluid restriction

Table 6. Mean (\pm SD) urinary output and frequency of micturition in a double-blind crossover study of 25 female patients with nocturia treated with desmopressin (reproduced with permission)^[80]

| | Baseline | Placebo | Desmopressin |
|--|---------------|----------------|----------------|
| Nocturnal urinary output (ml) | 438 \pm 183 | 391 \pm 181 | 267 \pm 97 |
| Nocturnal frequency of micturition (episodes per night) | 3.2 \pm 1.4 | 2.6 \pm 1.6 | 1.9 \pm 1.2 |
| Diurnal urinary output (ml) | 789 \pm 194 | 707 \pm 399 | 879 \pm 155 |
| Diurnal frequency of micturition (episodes per 24 hours) | 9.2 \pm 3.7 | 10.1 \pm 4.0 | 10.2 \pm 4.3 |

Desmopressin therapy was found to be effective in 50% of 18 evaluable patients. However, the investigators also reported a significant drop in serum sodium concentration (141 to 137 mmol/L) and concluded that close monitoring of patients (in terms of fluid intake) and their serum sodium levels may be necessary in desmopressin-treated nocturia.^[83]

Oral desmopressin

Oral desmopressin (200–800 µg/day) was found to be an effective, well-tolerated treatment for daytime urinary incontinence in patients with multiple sclerosis.^[77]

A double-blind, crossover study of 17 elderly patients with nocturia compared desmopressin (up to 400 µg each night) and placebo.^[9] After 14 days' treatment, a decrease (from baseline) in mean nocturnal urinary output was observed with desmopressin therapy compared with placebo (–0.7 and –0.1 ml/min, respectively). As expected, the duration of sleep between micturitions increased by around 2 hours during desmopressin therapy.

IMPORTANT: The latter conditions are not among those for which desmopressin is presently registered. Further clinical trials will reveal information about which specific patient groups are likely to benefit from antidiuretic treatment.

Renal concentrating capacity test (RCCT)

In a number of renal diseases, tubular function is reduced before glomerular function is

affected. A reduced tubular function is reflected by a reduced capacity of the kidney to concentrate the urine, i.e. by a reduced response to vasopressin. To this end, the water deprivation test, with or without injection of Pitressin® (vasopressin) is widely used for measuring renal concentrating capacity.^[84,85] However, water deprivation for 16–24 hours is inconvenient and even harmful to already dehydrated patients. In addition, misleading results can sometimes be obtained as a result of non-compliance with test protocol, e.g. if the patient has been drinking during the test. Therefore, it is more rational to stimulate the renal V₂ receptors directly with an exogenous V₂ agonist such as desmopressin. Administration of vasopressin will stimulate renal concentrations of urine in the same manner as desmopressin, but the latter agent is preferable as it is devoid of the intestinal and vascular constricting effects of vasopressin.

This was substantiated by Aronson and Svenningsen, who introduced desmopressin for testing renal concentrating ability in children and found it advantageous in comparison with both water deprivation and Pitressin®.^[86]

Desmopressin (Minirin®, DDAVP®) followed by a test of urine osmolality is a reliable and simple method for estimating renal concentrating capacity

There are four main indications for testing the renal capacity to concentrate urine:

- urinary tract infection
- polyuria/polydipsia
- lithium treatment
- analgesic-induced renal dysfunction.

Application of the RCCT

One hour after desmopressin administration, the bladder is emptied; urine osmolality is determined in two samples 3—5 h later.^[87] A number of age-adjusted reference values (lowest acceptable maximum urine osmolality) have been adopted for renal concentrating capacity following the administration of desmopressin (table 7; fig. 6). In general, if the maximum osmolality is <700 mOsm/kg the renal concentrating ability is considered

Table 7. Mean age-adjusted reference values for renal concentrating capacity following intranasal desmopressin 40 µg or subcutaneous desmopressin 4 µg

| Age (years) | Lowest acceptable maximum urine osmolality (mOsm/kg) |
|-------------|--|
| 1 | 525 ^a |
| 3 | 825 ^a |
| 20 | 850 ^b |
| 40 | 800 ^b |
| 60 | 700 ^b |
| 80 | 600 ^b |

^a Based on tests in 473 healthy volunteers.^[87]

^b Based on tests in 225 healthy volunteers.^[88]

abnormal.^[89] Fluid restriction is not considered necessary for accurate test results, but there is a risk of water intoxication with excessive fluid intake.^[90]

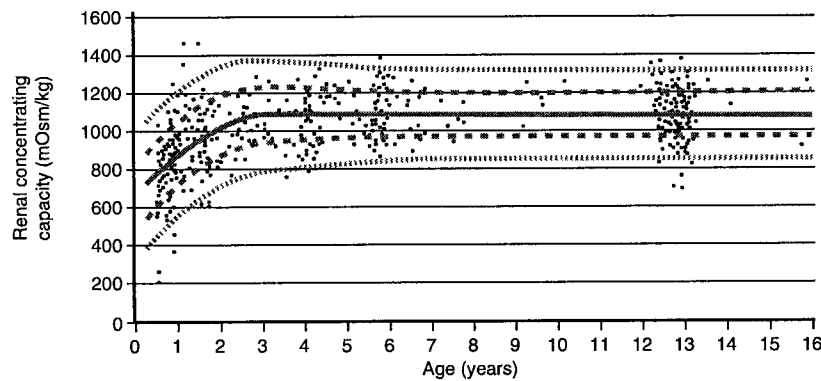


Fig.6. Reference curve for renal concentrating capacity for 0.5- to 14-year-old children as determined by the desmopressin test. Continuous lines represent mean values and broken lines represent intervals of one SD (© 1997 Springer-Verlag GmbH & Co. KG, reproduced with permission).^[87]

Indications for the RCCT

Urinary tract infections

Concentrating capacity is the first parameter of renal function to be impaired in children with chronic pyelonephritis. Thus, the RCCT provides a simple and practical method for the estimation of renal impairment in such patients. It is also recommended in the follow-up of children with urinary tract infection.^[91] In addition, desmopressin has been used for 10 years in Scandinavia to determine the extent of urinary tract infection. Normal concentrating capacity indicates that the infection is limited to the lower urinary tract, whereas a reduced concentrating capacity suggests that the kidneys are involved.

Investigation of polydipsia/polyuria

The RCCT can be used to distinguish between psychogenic polydipsia and the two forms of diabetes insipidus. A considerable reduction in renal concentrating capacity is seen in nephrogenic diabetes insipidus, a moderate reduction indicating psychogenic polydipsia and a normal renal concentrating capacity being observed in patients with central diabetes insipidus.

Detection of renal impairment in patients receiving lithium

In a small proportion of patients receiving long-term lithium treatment there exists a partly irreversible reduction in distal and collecting tubule function,^[92] and thus a reduced capacity to concentrate urine.^[93] There may also be reductions in vasopressin secretion. The desmopressin RCCT is a suitable method for testing renal concentrating capacity in lithium recipients and is as effective as fluid deprivation.^[94] Tubular function of 124 lithium-treated patients, as measured with the desmopressin RCCT, was below normal in 51% of patients in a study by Bendz et al.^[95] However, glomerular function was below normal in only 3% of patients, indicating that the RCCT is a more sensitive test of renal dysfunction than a test of glomerular function.

Early detection of renal dysfunction caused by analgesics

Around 5% of patients with terminal renal insufficiency have analgesic nephropathy, and the RCCT has proved useful in the early diagnosis of renal dysfunction caused by analgesics.^[96]

CLINICAL SAFETY

Clinical experience has shown desmopressin (Minirin®, DDAVP®) to be safe and well tolerated. During the 20 years of clinical experience with desmopressin, few adverse events have been reported when the drug is used in accordance with the manufacturer's recommendations. Besides infrequent cases of water intoxication, desmopressin is virtually free of serious adverse effects^[36] and has been associated with no abnormalities in routine laboratory tests.^[60,66,69] Among the non-serious adverse drug reactions experienced with desmopressin, nasal symptoms dominate. There are also occasional gastrointestinal symptoms, including nausea and abdominal pain.

Clinical experience has shown desmopressin (Minirin®, DDAVP®) to be well tolerated

Acute water intoxication is a rare complication of desmopressin therapy and is likely to occur only in patients who fail to reduce their water intake.^[97]

To reduce the risk of fluid retention, desmopressin (Minirin®, DDAVP®) recipients should control their fluid intake

Although patients could be instructed to limit fluid intake to no more than 30 ml/kg for a period of 2 hours before to 12 hours after taking desmopressin,^[98] a more practical recommendation might be to abstain from fluid intake for 8 hours after the medication or to take fluid only for the purpose of satisfying thirst.

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ORIGINAL ARTICLES

Successful transdermal administration of therapeutic doses of a polypeptide to normal human volunteers

The human stratum corneum constitutes a relatively impermeable barrier to the transdermal absorption of most substances, including polypeptides and proteins. This double-blind, randomized, crossover study in 13 normal men evaluated whether a low level of electrical current could induce changes in cutaneous permeability sufficient to produce absorption of a polypeptide. We compared cutaneous absorption of 5 mg of leuprolide (a 9 amino acid luteinizing hormone releasing hormone analogue) in transdermal patches containing 0.2 mA electrical current (active) and in patches containing no electrical current (passive). Serum luteinizing hormone (LH) concentration was measured 12 times during an 8-hour period as a measure of drug effect. Similar baseline LH levels were seen in each group: active = 11.3 ± 3.1 mIU/ml and passive = 13.7 ± 4.7 mIU/ml (p not significant). Significant elevations of LH were seen in active compared with passive patches ($p = 0.0084$). As predicted, passive patches produced no elevation of LH concentration (LH = 11.8 ± 7.1 mIU/ml at 4 hours). However, active patches produced elevations comparable to those achieved with subcutaneous administration of the drug (LH = 56.4 ± 49.6 mIU/ml at 4 hours and $p = 0.003$ compared with passive). The patches were well tolerated without significant cutaneous toxicity. It is concluded that the use of low levels of electrical current can induce changes in the permeability of the stratum corneum. These changes are sufficient to promote the transdermal absorption of therapeutically relevant amounts of a polypeptide. This has major importance for our understanding of skin permeability and for the development of new techniques for drug administration. (CLIN PHARMACOL THER 1988;44:607-12.)

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The human skin is a complicated and multifunctional organ. It is a chemical barrier separating the external environment and the internal organs of the individual, a mechanical barrier to the entrance of microorganisms, the site of thermoregulation, a sensory end organ, and an important endocrine organ.¹ The barrier function of the skin is located in the stratum corneum of the epidermis. This layer is 15 to 20 cells thick and is resistant to penetration by most chemical compounds. Although there has been investigation into the permeability characteristics of the stratum corneum and the role of extracellular lipids in the maintenance of this barrier, our understanding of the mechanisms by which the barrier function is maintained remains incomplete.²⁻⁹

An increased appreciation of the potential advantages of the transdermal route of administration for drugs has led to renewed interest in this area. Transdermal administration of medication may improve the serum concentration-time profile of medications with narrow therapeutic windows, thereby limiting toxicity and improving efficacy. It may provide greater ease of administration and improve patient compliance. It may also provide the only practical means for outpatient administration of a variety of medications with extensive gastrointestinal degradation or hepatic first-pass metabolism.¹⁰⁻¹²

The problem of oral administration is particularly dramatic in the case of polypeptides or proteins, where the only currently available technique for administration is parenteral administration by injection or intravenous infusion. Attempts to develop alternative routes of administration (principally nasal or buccal administration) have been undertaken. To date, success with these routes has been limited. Only one compound (DDAVP) is currently available in such a delivery system.¹³

We undertook this study to evaluate the effect of low levels of electrical current on the barrier function of the skin and to assess the effect of this current on the transdermal absorption of a polypeptide. Because of its safety, and the ease of assessing appropriate biologic effect, we chose to use leuprolide (Lupron) as a model polypeptide for this study.

METHODS

This study was designed as a double-blind crossover study. Normal male volunteers were studied on 2 days, 1 week apart. On the first study day, subjects were randomized to receive either a patch containing electrical current (active patch) or an identical-appearing patch that did not contain electrical current (passive patch). On the second study day, subjects were crossed over to receive the opposite arm of the study.

Volunteers were required to provide a complete medical history and to undergo a routine physical examination. Exclusion criteria for the study included the presence of any malignancy other than localized cutaneous malignancies, a history of major cardiovascular disease, significant pathologic dysfunction of any organ system, a history of sexual dysfunction, pituitary disease, or significant cutaneous disease. Before acceptance to the study all volunteers were also required to obtain a complete blood count, biochemical profile, urinalysis, and ECG. All volunteers were required to give written, informed consent for participation in the study.

This study was conducted under an IND granted by the Food and Drug Administration and was approved by the institutional review board of North Shore University Hospital.

All patches were prepared by Drug Delivery Systems Inc. The patches are approximately 70 cm² and contain an intrinsic power source supplied by small disk batteries, a resistance system to limit current, electronic conditioning components, and two drug reservoirs. Patches containing electrical current have one drug reservoir at the positive and one at the negative electrode. On application to the skin, the electrical circuit is completed. Active patches designed to deliver 0.2 mA current were provided. The batteries provided 9V. Passive patches were identical in design with the exception that appropriate wiring connections to complete the electrical circuit were not made. The two types of patches were indistinguishable on routine visual examination.

Patches were provided to the investigators in envelopes containing two patches (one active and one passive). Each patch was numbered, and the envelope specified one patch as being for study day 1 and the other as being for study day 2. One half of the envelopes contained active patches for study day 1 and one half contained active patches for study day 2. The investigators were not aware of the order of study for any particular envelope. After receiving the envelopes from Drug Delivery Systems, one of the investigators (B. R. M.) randomly assigned each envelope to a particular volunteer. The code was not broken until the completion of the study.

On the day of the study the patches to be used that day were removed from their envelopes and prepared as follows: (1) 1 ml (5 mg) of commercially available leuprolide solution (Lupron, 5 mg/ml) purchased from TAP Pharmaceuticals (North Chicago, Ill.) was added to the reservoir of the positive electrode; (2) 3 ml sodium phosphate buffering solution was added to the reservoir of the negative electrode.

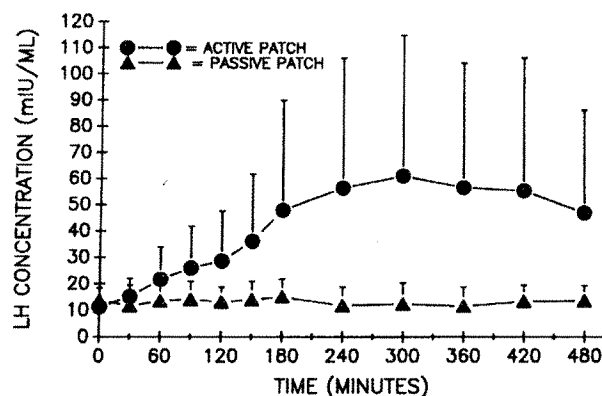


Fig. 1. Mean serum LH concentrations in active vs passive patches.

On the day of study routine vital signs were obtained in all subjects followed by the placement of a heparin lock. After obtaining baseline blood specimens for the determination of luteinizing hormone (LH) and testosterone levels, a patch was applied to the volar surface of the contralateral arm in each subject. Care was taken to avoid any area of skin with abrasions or other evidence of disruption of normal skin architecture. All patches were held in place by a peripheral coating of tape and by wrapping with a gauze or ace bandage.

After application of the patch, specimens of blood were obtained from all subjects through the heparin lock 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, and 480 minutes after application. Specimens were allowed to clot and were then centrifuged. Serum was then aspirated and frozen immediately for subsequent determination of LH and testosterone levels. After the completion of the final blood drawing, the patch was removed and the skin examined for evidence of injury from the patch.

Serum was subsequently analyzed in the central Nuclear Medicine Research Laboratory at North Shore University Hospital for serum LH and serum testosterone concentrations. Serum LH concentrations were determined with the Amerlex LH RIA kit (Amersham Corp, Arlington Heights, Ill.). This assay has a sensitivity of 2.0 mIU/ml. Intraassay coefficient of variation is 4.6%, and interassay coefficient of variation is 10.2%. Cross-reactivity with thyroid-stimulating hormone is 3.8%. Serum testosterone concentrations were determined with the DSL testosterone kit (Diagnostic

Systems Laboratories, Webster, Texas). The assay has a lower limit of sensitivity of 0.02 ng/ml and intraassay and interassay coefficients of variation of 8.14% and 8.09%, respectively. Cross-reactivity with dihydrotestosterone is 8.6%, and androstenedione reactivity is 1.6%. Counting was performed on a Packard Minaxi 5000 gamma-counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

Statistical analysis. Data were analyzed by a repeated-measures ANOVA with SAS software operating on an IBM PC. Data at individual time points were also analyzed by a paired *t* test for the comparison of means. Because of the performance of multiple comparisons, a level of significance of 0.005 (one tailed) was required.

RESULTS

Thirteen normal human volunteers were successfully recruited for the study. All subjects who began the study completed it successfully. After the termination of the study, one active patch was noted to have cracking of the electrode and subsequent loss of electrical current. All other patches appeared to be functional.

Serum LH concentrations. Mean serum LH concentrations for all subjects are shown in Table 1 and Fig. 1. Serum LH concentrations with active patches were elevated significantly compared with those of passive controls by 120 minutes ($p < 0.005$) and remained significantly elevated for the duration of the study. Values obtained at 60 and 90 minutes closely approached statistical significance, suggesting that therapeutic effect may even be seen at this point. Passive patches did

Table I. Mean serum LH concentrations

| Time (min) | Passive patches (mU/ml \pm SD) | Active patches (mU/ml \pm SD) | Significance |
|------------|----------------------------------|---------------------------------|--------------|
| 0 | 13.7 \pm 4.7 | 11.3 \pm 3.1 | NS |
| 30 | 11.7 \pm 7.8 | 15.1 \pm 7.0 | NS |
| 60 | 13.5 \pm 7.5 | 21.7 \pm 12.3 | 0.028 |
| 90 | 14.1 \pm 6.9 | 26.0 \pm 15.8 | 0.010 |
| 120 | 13.1 \pm 5.7 | 28.7 \pm 18.9 | 0.004 |
| 150 | 14.0 \pm 7.0 | 36.1 \pm 25.6 | 0.003 |
| 180 | 15.0 \pm 6.7 | 47.9 \pm 42.0 | 0.005 |
| 240 | 11.8 \pm 7.1 | 56.4 \pm 49.6 | 0.003 |
| 300 | 12.4 \pm 8.0 | 61.0 \pm 53.7 | 0.002 |
| 360 | 11.6 \pm 7.3 | 56.6 \pm 47.5 | 0.001 |
| 420 | 13.4 \pm 6.2 | 55.4 \pm 50.8 | 0.003 |
| 480 | 13.6 \pm 5.8 | 47.0 \pm 39.3 | 0.003 |

ANOVA (active vs passive), all cases, $p = 0.0084$.ANOVA (active vs passive), case 7 excluded, $p = 0.0060$.

not produce a significant elevation above baseline LH levels.

ANOVA showed very highly significant differences between active and passive patches, with $p = 0.0084$. This analysis included the single subject in whom cracking of the electrode was noted. If this subject was excluded from the analysis, the values became even more significant ($p = 0.0060$).

Serum testosterone concentrations. Mean serum testosterone concentrations at each time point for both active and passive patches are shown in Table II. No significant differences between active and passive patches were seen.

Toxicity. All subjects tolerated the study well. Three individuals (two active and one passive) noted the presence of a mild tingling sensation over the area of the patch. In one case the presence of this sensation led the individual to request that his patch be removed 10 minutes before the completion of the study. No evidence of cutaneous injury was noted at the time of patch removal.

The skin under both active and passive patches appeared to be well hydrated, although this was more so in the active than in the passive patches. Mild erythema at the site of the patch was noted in six of 13 subjects while receiving the active patches and in one of 13 subjects while receiving the passive patches. No subject had any evidence of blistering, burning, or other significant cutaneous damage. In all circumstances this erythema resolved rapidly without sequelae.

DISCUSSION

The skin serves as an effective barrier between the external environment and the internal organ systems of

Table II. Mean serum testosterone levels

| Time (min) | Passive system (ng/dl) | Active system (ng/dl) |
|------------|------------------------|-----------------------|
| 0 | 643 \pm 228 | 609 \pm 182 |
| 30 | 553 \pm 154 | 539 \pm 157 |
| 60 | 498 \pm 174 | 512 \pm 134 |
| 90 | 493 \pm 144 | 497 \pm 131 |
| 120 | 511 \pm 130 | 530 \pm 137 |
| 150 | 508 \pm 155 | 543 \pm 149 |
| 180 | 557 \pm 154 | 554 \pm 163 |
| 240 | 499 \pm 115 | 561 \pm 173 |
| 300 | 473 \pm 171 | 541 \pm 152 |
| 360 | 474 \pm 167 | 516 \pm 160 |
| 420 | 458 \pm 154 | 531 \pm 191 |
| 480 | 447 \pm 120 | 533 \pm 151 |

Data are mean \pm SD.

the body. The principal location of this barrier function is in the most superficial layer of the epidermis, the stratum corneum. Gradual stripping away of the stratum corneum produces gradual increase in skin permeability.^{2,3} The stratum corneum has a relatively low water content (approximately 20%) and is rich in both intracellular and extracellular lipids.¹² Diseases that specifically alter the lipid content of the stratum corneum produce alterations in transdermal absorption, which reverse with correction of the deficit.^{8,9}

The potential advantages of transdermal administration of medication have been well recognized, and several transdermal medications have been developed. Nonetheless, the characteristics of the stratum corneum have limited the transdermal administration of medications to a relatively small number of molecules having excellent potency (necessitating the delivery of only small amounts of medication for therapeutic effect), excellent solubility in both oil and water, and a relatively large and constant toxic-therapeutic ratio.¹² Attempts to alter the stratum corneum by the use of enhancers such as Azone or the esters of long-chain fatty acids have produced improvements in transdermal absorption but have not yet provided the predictability, reproducibility, and safety that allow extensive clinical use.¹⁴⁻¹⁶

The use of an electrical current to induce transdermal absorption of a drug has been investigated previously. Attempts to use iontophoresis have not been widely successful. The levels of current involved have been prohibitively large, and the compounds studied have of necessity been highly polar molecules.^{17,18} The use of lower levels of electrical current (below those used in iontophoresis) has been reported previously only in limited animal studies¹⁹ but has not been investigated in human beings.

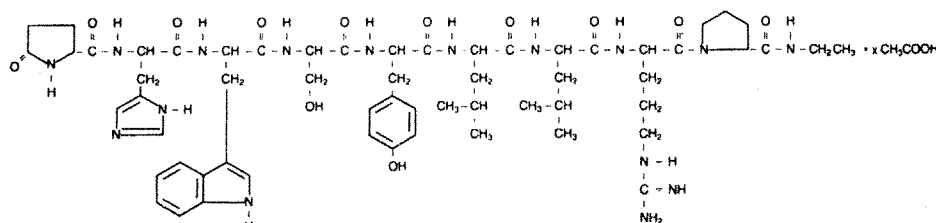


Fig. 2. Leuprolide acetate (molecular weight 1209.41); octanol/water partition coefficient at pH 5 = 3.5×10^{-5}

The possibility of avoiding gastrointestinal degradation and hepatic first-pass metabolism makes the transdermal route particularly attractive for compounds with extensive degradation during oral administration. This is especially true for proteins and polypeptides, where parenteral administration by intravenous infusion or intermittent injection has been a necessity. Attempts to circumvent this problem by the use of alternative routes, including intranasal administration, have met with only limited success.¹³ As polypeptides and proteins become more important parts of our therapeutic armamentarium, it would be a major therapeutic advantage to have a technique for safe and effective transdermal administration.

Leuprolide is a useful agent for use in the evaluation of transdermal administration of peptides. It is a synthetic 9 amino acid luteinizing hormone releasing hormone (LH-RH) analogue (molecular weight 1209.41), differing from LH-RH by the presence of the D-isomer of leucine at position 6, and the presence of an ethylamide group in place of glycine at position 10 (see Fig. 2). The compound is extremely safe for single-dose administration,²⁰ and its biologic effect (the elevation of serum LH concentration) is rapid and easily measurable. The compound is approved in the United States for the hormonal treatment of metastatic prostatic carcinoma, where it is given by daily injection.²¹ Other potential uses for this class of compounds have included the management of benign prostatic hypertrophy,²² the treatment of infertility,²³ and contraception.²⁴ Most of these potential indications would require the development of an alternative to daily injection therapy.

This study clearly demonstrates the ability of low levels of electrical current to effectively alter stratum corneum permeability and to induce successful transdermal transport of leuprolide in normal human vol-

unteers. The elevations in serum LH concentrations seen with the active patches are significant as early as 120 minutes and may well be demonstrated to be significantly elevated above baseline as early as 30 minutes after patch application. The magnitude of the elevations of LH produced by the active patches is in a range that should be adequate for therapeutic effect of the drug and is comparable to that achieved by subcutaneous administration of the drug.

The variable magnitude of the LH response noted in the study, as evidenced by the large standard deviations, may reflect either variability in absorption of leuprolide or variability in LH response. If the three subjects with the most dramatic LH response are excluded from the study, the standard deviation becomes less dramatic, but the differences between active and passive patches remain significant ($p < 0.01$). In the absence of data concerning serum leuprolide concentrations, there is no definitive way to differentiate these possibilities. Unfortunately, an accurate technique for the determination of leuprolide concentrations is not generally available.

This study did not document any changes in serum testosterone concentration in either the active or passive systems. This is not surprising given the length of the study and the time course of testosterone response to elevation of LH.

In contrast to the active patches, no significant changes in serum LH concentration were seen with the passive patch system. This confirms prior wisdom that passive delivery of a polypeptide such as leuprolide across the skin is not achievable. The patches appeared to be well tolerated by the volunteers, as evidenced by the absence of significant injury to the skin, blistering, or other reaction suggesting injury.

The mechanism of the transdermal transport of leuprolide observed in this study needs further investigation. The level of electrical current used and the chem-

ical structure of leuprolide make it very unlikely that iontophoretic absorption is occurring. It seems most likely that the absorption occurs by the process of electroosmosis, in which the movement of water in an electrical current leads to the secondary flow of solutes. Further studies will clearly be necessary to elaborate this mechanism. Our study does not rule out the possibility that the electrical current itself caused the elevation of LH that we noted. However, we are aware of no evidence that local application of low levels of electrical current is able to induce a rise in LH. We therefore consider this possibility unlikely.

The current study constitutes the first report of the transdermal administration of therapeutic doses of a polypeptide to humans. It provides new information concerning the process of transdermal absorption and raises exciting new possibilities for advances in pharmacologic therapeutics.

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RELATED PROCEEDINGS APPENDIX

None.